Appendices

A Theoretical details for thresholding estimator

A.1 Proposition statements

This subsection contains detailed proposition statements for the informal claims made in Section 4.2. For simplicity we derive results in the case $\pi = 1/2$. In reality π is smaller; however, setting π to 1/2 simplifies the proofs substantially and enables us to recover the most interesting and important statistical phenomena (e.g., attenuation bias, monotonic impact of β_1^g on problem difficulty, etc.). We state state five propositions labeled 3 – 7 corresponding to the informal claims made in Section 4.2; these propositions visually are depicted in Figure 6.

First, the thresholding method incurs strict attenuation bias (i.e., it *under*estimates the true effect size) for all choices of the threshold and over all possible values of the model parameters:

Proposition 3. *Fix $\pi = 1/2$. For all $(\beta_1^g, c, \beta_0^g) \in \mathbb{R}^3$, the asymptotic relative bias is positive, i.e.*

$$b(\beta_1^g, 1/2, c, \beta_0^g) > 0.$$

Next, the asymptotic relative bias b decreases monotonically in β_1^g :

Proposition 4. *Fix $\pi = 1/2$. The asymptotic relative bias b decreases monotonically in β_1^g, i.e.*

$$\frac{\partial b}{\partial(\beta_1^g)}(\beta_1^g, 1/2, c, \beta_0^g) \leq 0.$$

Let c_{bayes} denote the Bayes-optimal decision boundary for classifying cells as perturbed or unperturbed, i.e. $c_{\text{bayes}} = (1/2)(\beta_0^g + \beta_1^g)$ for $\pi = 1/2$. We have that c_{bayes} is a critical value of the bias function.

Proposition 5. *For $\pi = 1/2$ and given $(\beta_1^g, \beta_0^g) \in \mathbb{R}^2$, the Bayes-optimal decision boundary c_{bayes} is a critical value of the bias function b, i.e.*

$$\frac{\partial b}{\partial c}(\beta_1^g, 1/2, c_{bayes}, \beta_0^g) = 0.$$

Furthermore, as the threshold tends to infinity, the asymptotic relative bias b tends to π.

Proposition 6. *Assume without loss of generality that $\beta_1^g > 0$. As the threshold c tends to infinity, the asymptotic relative bias b tends to π, i.e.*

$$\lim_{c\to\infty} b(\beta_1^g, \pi, c, \beta_0^g) = \pi.$$

As a corollary, when $\pi = 1/2$, asymptotic relative bias tends to $1/2$ as c tends to infinity. Finally, we compare two threshold selection strategies head-to-head: setting the threshold to an arbitrarily large number, and setting the threshold to the Bayes-optimal decision boundary.

Proposition 7. *Assume without loss of generality that $\beta_1^g > 0$. For $\beta_1^g \in [0, 2\Phi^{-1}(3/4))$, we have that*

$$b(\beta_1^g, 1/2, c_{bayes}, \beta_0^g) > b(\beta_1^g, 1/2, \infty, \beta_0^g).$$

For $\beta_1^g = 2\Phi^{-1}(3/4)$, we have that

$$b(\beta_1^g, 1/2, c_{bayes}, \beta_0^g) = b(\beta_1^g, 1/2, \infty, \beta_0^g).$$

Finally, for $\beta_1^g \in (2\Phi^{-1}(3/4), \infty)$*, we have that*

$$b(\beta_1^g, 1/2, c_{bayes}, \beta_0^g) < b(\beta_1^g, 1/2, \infty, \beta_0^g).$$

In other words, setting the threshold to a large number yields a smaller bias when β_1^g is small (i.e., $\beta_1^g < 2\Phi^{-1}(3/4) \approx 1.35$; Figure 7a, left); setting the threshold to the Bayes-optimal decision boundary yields a smaller bias when β_1^g is large (i.e., $\beta_1^g > 2\Phi^{-1}(3/4)$; Figure 7a, right); and the two approaches coincide when β_1^g is intermediate (i.e., $\beta_1^g = 2\Phi^{-1}(3/4)$; Figure 7a, middle).

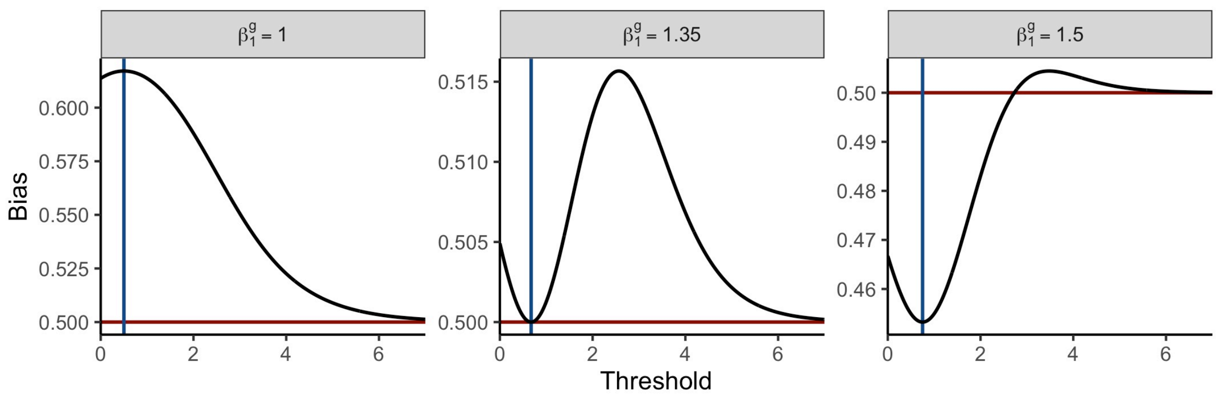

Figure 6: **Bias as a function of threshold**. This figure visually depicts Propositions 3-7, which were stated informally in the main text. Asymptotic relative bias is plotted on the vertical axis, and the threshold is plotted on the horizontal axis. Panels correspond to different values of β_1^g. Vertical blue lines indicate the Bayes-optimal decision boundary. Observe that (a) bias is strictly nonzero (proposition 3); (b) bias decreases monotonically in β_1^g (Proposition 4); (c) the Bayes-optimal decision boundary is a critical value of the bias function (Proposition 5), in some cases a maximum and in other cases a minumum; (d) as the threshold tends to infinity, the bias converges to 1/2 (Proposition 6); and (e) when $\beta_1^g < 1.35$, an arbitrarily large number yields a smaller bias; by contrast, when $\beta_1^g > 1.35$, the Bayes-optimal decision boundary yields a smaller bias (Proposition 7).

A.2 Organization

The following subsections prove all propositions. Section A.3 introduces some notation. Section A.4 establishes almost sure convergence of the thresholding estimator in the model

(5), proving Proposition 1. Section A.5 simplifies the expression for the attenuation function γ, and section A.6 computes derivatives of γ to be used throughout the proofs. Section A.7 establishes the limit in c of γ, proving Proposition 6. Section A.8 establishes that the Bayes-optimal decision boundary is a critical value of γ, proving Proposition 5, and section A.9 compares the competing threshold selection strategies head-to-head, proving Proposition 7. Section A.10 demonstrates that γ is monotone in β_1^g, proving Proposition 4, and Section A.11 establishes attenuation bias of the thresholding estimator, proving Proposition 3. Finally, Section A.12 derives the bias-variance decomposition of the thresholding estimator in the no-intercept version of 5, proving Proposition 2.

A.3 Notation

All notation introduced in this subsection (i.e., A.3) pertains to the Gaussian model with intercepts (5). Recall that the attenuation function $\gamma : \mathbb{R}^4 \to \mathbb{R}$ is defined by

$$\gamma(\beta_1^g, c, \pi, \beta_0^g) = \frac{\pi(\omega - \mathbb{E}[\hat{p}_i])}{\mathbb{E}[\hat{p}_i](1 - \mathbb{E}[\hat{p}_i])},$$

where

$$\mathbb{E}[\hat{p}_i] = \zeta(1-\pi) + \omega\pi; \quad \omega = \Phi\left(\beta_1^g + \beta_0^g - c\right); \quad \zeta = \Phi\left(\beta_0^g - c\right).$$

Additionally, recall that the asymptotic relative bias function $b : \mathbb{R}^4 \to \mathbb{R}$ is $b(\beta_1^g, c, \pi, \beta_0^g) = 1 - \gamma(\beta_1^g, c, \pi, \beta_0^g)$. Next, we define the functions g and $h : \mathbb{R}^4 \to \mathbb{R}$ by

$$g(\beta_1^g, c, \pi, \beta_0^g) = (1-\pi)\left(\Phi(\beta_0^g + \beta_1^g - c)\right) - (1-\pi)\left(\Phi(\beta_0^g - c)\right) \tag{11}$$

and

$$h(\beta_1^g, c, \pi, \beta_0^g) = \left[(1-\pi)\left(\Phi(\beta_0^g - c)\right) + \pi\left(\Phi(\beta_0^g + \beta_1^g - c)\right)\right] \times$$
$$\left[(1-\pi)\left(\Phi(c - \beta_0^g)\right) + \pi\left(\Phi(c - \beta_0^g - \beta_1^g)\right)\right]. \quad (12)$$

We use $f : \mathbb{R} \to \mathbb{R}$ to denote the $N(0,1)$ density, and we denote the right-tail probability probability of f by $\bar{\Phi}$, i.e.,

$$\bar{\Phi}(x) = \int_x^\infty f = \Phi(-x).$$

The parameter β_0^g is a given, fixed constant throughout the proofs. Therefore, to minimize notation, we typically use $\gamma(\beta_1^g, c, \pi)$ (resp., $b(\beta_1^g, c, \pi)$, $g(\beta_1^g, c, \pi)$, $h(\beta_1^g, c, \pi)$) to refer to the function γ (resp., b, g, h) evaluated at $(\beta_1^g, c, \pi, \beta_0^g)$. Finally, for a given function $r : \mathbb{R}^p \to \mathbb{R}$, point $x \in \mathbb{R}^p$, and index $i \in \{1, \ldots, p\}$, we use the symbol $D_i r(x)$ to refer to the derivative of the ith component of r evaluated at x (*sensu* Fitzpatrick 2009). For example, $D_1\gamma(\beta_1^g, c, 1/2)$ is the derivative of the first component of γ (the component corresponding to β_1^g) evaluated at $(\beta_1^g, c, 1/2)$. Likewise, $D_2 g(\beta_1^g, c, \pi)$ is the derivative of the second component of g (the component corresponding to c) evaluated at (β_1^g, c, π).

A.4 Almost sure limit of $\hat{\beta}_1^m$

We derive the limit in probability of $\hat{\beta}_1^m$ for the Gaussian model with intercepts (5). Dividing by n in (6), we can express $\hat{\beta}_1^m$ as

$$\hat{\beta}_1^m = \frac{\frac{1}{n}\sum_{i=1}^n (\hat{p}_i - \overline{\hat{p}_i})(m_i - \overline{m})}{\frac{1}{n}\sum_{i=1}^n (\hat{p}_i - \overline{\hat{p}})}.$$

By weak LLN, $\hat{\beta}_1^m \xrightarrow{P} \text{Cov}(\hat{p}_i, m_i)/\mathbb{V}\left(\hat{p}_i\right)$. To compute this quantity, we first compute several simpler quantities:

1. Expectation of m_i: $\mathbb{E}[m_i] = \beta_0^m + \beta_1^m \pi$.

2. Expectation of $\hat{p}_i$:

$$\mathbb{E}[\hat{p}_i] = \mathbb{P}\left[\hat{p}_i = 1\right] = \mathbb{P}\left[\beta_0^g + \beta_1^g p_i + \tau_i \geq c\right] =$$

$$\text{(By LOTP) } \mathbb{P}\left[\beta_0^g + \tau_i \geq c\right]\mathbb{P}\left[p_i = 0\right] + \mathbb{P}\left[\beta_0^g + \beta_1^g + \tau_i \geq c\right]\mathbb{P}[p_i = 1]$$

$$= \mathbb{P}\left[\tau_i \geq c - \beta_0^g\right](1-\pi) + \mathbb{P}\left[\tau_i \geq c - \beta_1^g - \beta_0^g\right](\pi)$$

$$= \left(\bar{\Phi}(c - \beta_0^g)\right)(1-\pi) + \left(\bar{\Phi}(c - \beta_1^g - \beta_0^g)\right)(\pi) =$$

$$\Phi(\beta_0^g - c)(1-\pi) + \Phi(\beta_1^g + \beta_0^g - c)\pi = \zeta(1-\pi) + \omega\pi.$$

3. Expectation of $\hat{p}_i p_i$: $\mathbb{E}\left[\hat{p}_i p_i\right] = \mathbb{E}\left[\hat{p}_i | p_i = 1\right]\mathbb{P}\left[p_i = 1\right] = \mathbb{P}\left[\beta_0^g + \beta_1^g + \tau_i \geq c\right]\pi = \omega\pi.$

4. Expectation of $\hat{p}_i m_i$:

$$\mathbb{E}\left[\hat{p}_i m_i\right] = \mathbb{E}[\hat{p}_i(\beta_0^m + \beta_1^m p_i + \epsilon_i)] = \beta_0^m \mathbb{E}\left[\hat{p}_i\right] + \beta_1^m \mathbb{E}\left[\hat{p}_i p_i\right] + \mathbb{E}[\hat{p}_i \epsilon_i]$$

$$= \beta_0^m \mathbb{E}[\hat{p}_i] + \beta_1^m \omega\pi + \mathbb{E}[\hat{p}_i]\mathbb{E}[\epsilon_i] = \beta_0^m \mathbb{E}[\hat{p}_i] + \beta_1^m \omega\pi.$$

5. Variance of $\hat{p}_i$: Because $\hat{p}_i$ is binary, we have that $\mathbb{V}[\hat{p}_i] = \mathbb{E}[\hat{p}_i]\left(1 - \mathbb{E}[\hat{p}_i]\right)$.

6. Covariance of $\hat{p}_i, m_i$:

$$\text{Cov}\left(\hat{p}_i, m_i\right) = \mathbb{E}\left[\hat{p}_i m_i\right] - \mathbb{E}[\hat{p}_i]\mathbb{E}[m_i] = \beta_0^m \mathbb{E}[\hat{p}_i] + \beta_1^m \omega\pi - \mathbb{E}[\hat{p}_i](\beta_0^m + \beta_1^m \pi)$$

$$= \beta_1^m \omega\pi - \mathbb{E}[\hat{p}_i]\beta_1^m \pi = \beta_1^m \pi\left(\omega - \mathbb{E}[\hat{p}_i]\right).$$

Combining these expressions, we have that

$$\hat{\beta}_1^m \xrightarrow{P} \frac{\beta_1^m \pi(\omega - \mathbb{E}[\hat{p}_i])}{\mathbb{E}[\hat{p}_i](1 - \mathbb{E}[\hat{p}_i])} = \beta_1^m \gamma(\beta_1^g, c, \pi).$$

A.5 Re-expressing γ in a simpler form

We rewrite the attenuation fraction γ in a way that makes it more amenable to theoretical analysis. We leverage the fact that f integrates to unity and is even. We have that

$$\mathbb{E}\left[\hat{p}_i\right] = (1-\pi)\bar{\Phi}(c-\beta_0^g) + \pi\bar{\Phi}(c-\beta_0^g-\beta_1^g) = (1-\pi)\Phi(\beta_0^g-c) + \pi\Phi(\beta_0^g+\beta_1^g-c), \tag{13}$$

and so

$$\begin{aligned} 1-\mathbb{E}\left[\hat{p}_i\right] &= (1-\pi)+\pi-\mathbb{E}[\hat{p}_i] = (1-\pi)\left(1-\bar{\Phi}(c-\beta_0^g)\right) + \pi\left(1-\bar{\Phi}(c-\beta_0^g-\beta_1^g)\right) \\ &= (1-\pi)\Phi(c-\beta_0^g) + \pi\Phi(c-\beta_0^g-\beta_1^g). \end{aligned} \tag{14}$$

Next,

$$\omega = \Phi(\beta_1^g+\beta_0^g-c), \tag{15}$$

and so

$$\begin{aligned} \omega-\mathbb{E}[\hat{p}_i] &= \Phi(\beta_1^g+\beta_0^g-c) - (1-\pi)\Phi(\beta_0^g-c) - \pi\Phi(\beta_0^g+\beta_1^g-c) \\ &(1-\pi)\Phi(\beta_1^g+\beta_0^g-c) - (1-\pi)\Phi(\beta_0^g-c). \end{aligned} \tag{16}$$

Combining (13, 14, 15, 16), we find that

$$\begin{aligned} \gamma(\beta_1^g, c, \pi) &= \frac{\pi(\omega-\mathbb{E}[\hat{p}_i])}{\mathbb{E}[\hat{p}_i](1-\mathbb{E}[\hat{p}_i])} \\ &= \frac{\pi\left[(1-\pi)\Phi(\beta_0^g+\beta_1^g-c) - (1-\pi)\Phi(\beta_0^g-c)\right]}{\left[(1-\pi)\Phi(\beta_0^g-c)+\pi\Phi(\beta_0^g+\beta_1^g-c)\right]\left[(1-\pi)\Phi(c-\beta_0^g)+\pi\Phi(c-\beta_0^g-\beta_1^g)\right]}. \end{aligned} \tag{17}$$

As a corollary, when $\pi = 1/2$,

$$\gamma(\beta_1^g, c, 1/2) = \frac{\Phi(\beta_0^g + \beta_1^g - c) - \Phi(\beta_0^g - c)}{\left[\Phi(\beta_0^g - c) + \Phi(\beta_0^g + \beta_1^g - c)\right]\left[\Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g)\right]}. \tag{18}$$

Recalling the definitions of g (11) and h (12), we can write γ as

$$\gamma(\beta_1^g, c, \pi) = \frac{\pi g(\beta_1^g, c, \pi)}{h(\beta_1^g, c, \pi)}.$$

The special case (18) is identical to

$$\gamma(\beta_1^g, c, 1/2) = \frac{(4)(1/2)g(\beta_1^g, c, 1/2)}{4h(\beta_1^g, c, 1/2)} = \frac{2g(\beta_1^g, c, 1/2)}{4h(\beta_1^g, c, 1/2)}, \tag{19}$$

i.e., the numerator and denominator of (19) coincide with those of (18). We sometimes will use the notation $2 \cdot g$ and $4 \cdot h$ to refer to the numerator and denominator of (18), respectively.

A.6 Derivatives of g and h in c

We compute the derivatives of g and h in c, which we will need to prove subsequent results. First, by FTC and the evenness of f, we have that

$$\begin{aligned} D_2 g(\beta_1^g, c, \pi) &= -(1-\pi) f(\beta_0^g + \beta_1^g - c) + (1-\pi) f(\beta_0^g - c) \\ &= (1-\pi) f(c - \beta_0^g) - (1-\pi) f(c - \beta_0^g - \beta_1^g). \end{aligned} \tag{20}$$

Second, we have that

$$D_2 h(\beta_1^g, c, \pi) = -[(1-\pi) f(\beta_0^g - c) + \pi f(\beta_0^g + \beta_1^g - c)]\,[(1-\pi)\Phi(c-\beta_0^g) + \pi\Phi(c-\beta_0^g-\beta_1^g)]$$

$$+\,[(1-\pi) f(c-\beta_0^g) + \pi f(c-\beta_0^g-\beta_1^g)]\,[(1-\pi)\Phi(\beta_0^g - c) + \pi\Phi(\beta_0^g+\beta_1^g-c)]$$

$$= [(1-\pi) f(c-\beta_0^g) + \pi f(c-\beta_0^g-\beta_1^g)] \times$$

$$\Big[(1-\pi)\Phi(\beta_0^g-c) + \pi\Phi(\beta_0^g+\beta_1^g-c) - (1-\pi)\Phi(c-\beta_0^g) - \pi\Phi(c-\beta_0^g-\beta_1^g)\Big]. \quad (21)$$

A.7 Limit of γ in c

Assume (without loss of generality) that $\beta_1^g > 0$. We compute $\lim_{c\to\infty} \gamma(\beta_1^g, c, \pi)$. Observe that

$$\lim_{c\to\infty} g(\beta_1^g, c, \pi) = \lim_{c\to\infty} h(\beta_1^g, c, \pi) = 0.$$

Therefore, we can apply L'Hôpital's rule. We have by (20) and (21) that

$$\lim_{c\to\infty} \gamma(\beta_1^g, c, \pi) = \lim_{c\to\infty} \frac{\pi D_2 g(\beta_1^g, c, \pi)}{D_2 h(\beta_1^g, c, \pi)}$$

$$= \lim_{c\to\infty} \left\{ \frac{(1-\pi) f(c-\beta_0^g) + \pi f(c-\beta_0^g-\beta_1^g)}{\pi(1-\pi) f(c-\beta_0^g) - \pi(1-\pi) f(c-\beta_0^g-\beta_1^g)} \times \right.$$

$$\left. \Big[(1-\pi)\Phi(\beta_0^g-c) + \pi\Phi(\beta_0^g+\beta_1^g-c) - (1-\pi)\Phi(c-\beta_0^g) - \pi\Phi(c-\beta_0^g-\beta_1^g)\Big] \right\}^{-1}. \quad (22)$$

We evaluate the two terms in the product (22) separately. Dividing by $f(c-\beta_0^g-\beta_1^g) > 0$, we see that

$$\frac{(1-\pi) f(c-\beta_0^g) + \pi f(c-\beta_0^g-\beta_1^g)}{\pi(1-\pi) f(c-\beta_0^g) - \pi(1-\pi) f(c-\beta_0^g-\beta_1^g)} = \frac{\frac{(1-\pi) f(c-\beta_0^g)}{f(c-\beta_0^g-\beta_1^g)} + \pi}{\frac{\pi(1-\pi) f(c-\beta_0^g)}{f(c-\beta_0^g-\beta_1^g)} - \pi(1-\pi)}. \quad (23)$$

To evaluate the limit of (23), we first evaluate the limit of

$$\frac{f(c-\beta_0^g)}{f(c-\beta_0^g-\beta_1^g)} = \frac{\exp[-(1/2)(c-\beta_0^g)^2]}{\exp[-(1/2)(c-\beta_0^g-\beta_1^g)^2]}$$

$$= \frac{\exp[-(1/2)(c^2 - 2c\beta_0^g + (\beta_0^g)^2)]}{\exp\left[-(1/2)(c^2 - 2c\beta_0^g - 2c\beta_1^g + (\beta_0^g)^2 + 2(\beta_0^g\beta_1^g) + (\beta_1^g)^2)\right]}$$

$$= \exp\Big[-c^2/2 + c\beta_0^g - (\beta_0^g)^2/2$$

$$+ c^2/2 - c\beta_0^g - c\beta_1^g + (\beta_0^g)^2/2 + \beta_0^g\beta_1^g + (\beta_1^g)^2/2\Big]$$

$$= \exp[-c\beta_1^g + \beta_0^g\beta_1^g + (\beta_1^g)^2/2] = \exp[\beta_0^g\beta_1^g + (\beta_1^g)^2/2]\exp[-c\beta_1^g]. \quad (24)$$

Taking the limit in (24), we obtain

$$\lim_{c\to\infty} \frac{f(c - \beta_0^g)}{f(c - \beta_0^g - \beta_1^g)} = \exp[\beta_0^g\beta_1^g + (\beta_1^g)^2/2] \lim_{c\to\infty} \exp[-c\beta_1^g] = 0$$

for $\beta_1^g > 0$. We now can evaluate the limit of (23):

$$\lim_{c\to\infty} \frac{(1-\pi)f(c-\beta_0^g) + \pi f(c-\beta_0^g-\beta_1^g)}{\pi(1-\pi)f(c-\beta_0^g) - \pi(1-\pi)f(c-\beta_0^g-\beta_1^g)} = \frac{-\pi}{\pi(1-\pi)} = -\frac{1}{1-\pi}.$$

Next, we compute the limit of the other term in the product (22):

$$\lim_{c\to\infty} \Big[(1-\pi)\Phi(\beta_0^g - c) + \pi\Phi(\beta_0^g + \beta_1^g - c)$$
$$- (1-\pi)\Phi(c-\beta_0^g) - \pi\Phi(c-\beta_0^g-\beta_1^g)\Big] = -(1-\pi) - \pi = -1. \quad (25)$$

Combining (23) and (25), the limit (22) evaluates to

$$\lim_{c\to\infty} \gamma(\beta_1^g, c, \pi) = \left(\frac{1}{1-\pi}\right)^{-1} = 1 - \pi.$$

It follows that the limit in c of the asymptotic relative bias b is

$$\lim_{c\to\infty} b(\beta_1^g, c, \pi) = 1 - \lim_{c\to\infty} \gamma(\beta_1^g, c, \pi) = \pi.$$

A corollary is that $\lim_{c\to\infty} b(\beta_1^g, c, 1/2) = 1/2$.

A.8 Bayes-optimal decision boundary as a critical value of γ

Let $c_{\text{bayes}} = \beta_0^g + (1/2)\beta_1^g$. We show that $c = c_{\text{bayes}}$ is a critical value of γ for $\pi = 1/2$ and given β_1^g, i.e, $D_2\gamma(\beta_1^g, c_{\text{bayes}}, 1/2) = 0$. Differentiating (19), the quotient rule implies that

$$D_2\gamma(\beta_1^g, c, 1/2) = \frac{D_2[2g(\beta_1^g, c, 1/2)]4h(\beta_1^g, c, 1/2) - 2g(\beta_1^g, c, 1/2)D_2[4h(\beta_1^g, c, 1/2)]}{[4h(\beta_1^g, c, \pi)]^2}. \quad (26)$$

We have by (20) that

$$D_2[2g(\beta_1^g, c_{\text{bayes}}, 1/2)] = f(\beta_1^g/2) - f(-\beta_1^g/2) = f(\beta_1^g/2) - f(\beta_1^g/2) = 0. \quad (27)$$

Similarly, we have by (21) that

$$D_2[4h(\beta_1^g, c_{\text{bayes}}, \pi)] = [f(\beta_1^g/2)+f(-\beta_1^g/2)]\,[\Phi(-\beta_1^g/2) + \Phi(\beta_1^g/2) - \Phi(\beta_1^g/2) - \Phi(-\beta_1^g/2)] = 0. \quad (28)$$

Plugging in (28) and (27) to (26), we find that $D_2[\gamma(\beta_1^g, c_{\text{bayes}}, 1/2)] = 0$. Finally, because

$$b(\beta_1^g, c, 1/2) = 1 - \gamma(\beta_1^g, c, 1/2),$$

it follows that

$$D_2[b(\beta_1^g, c_{\text{bayes}}, 1/2)] = -D_2[\gamma(\beta_1^g, c_{\text{bayes}}, 1/2)] = 0.$$

A.9 Comparing Bayes-optimal decision boundary and large threshold

We compare the bias produced by setting the threshold to a large number to the bias produced by setting the threshold to the Bayes-optimal decision boundary. Let $r : \mathbb{R}^{\geq 0} \to \mathbb{R}$ be the value of attenuation function evaluated at the Bayes-optimal decision boundary $c_{\text{bayes}} = \beta_0^g + (1/2)\beta_1^g$, i.e.

$$r(\beta_1^g) = \gamma(\beta_1^g, \beta_0^g + (1/2)\beta_1^g, 1/2) = \frac{\Phi(\beta_1^g/2) - \Phi(-\beta_1^g/2)}{[\Phi(-\beta_1^g/2) + \Phi(\beta_1^g/2)]\,[\Phi(\beta_1^g/2) + \Phi(-\beta_1^g/2)]}$$
$$= \frac{\int_{-\beta_1^g/2}^{\beta_1^g/2} f}{[1 - \Phi(\beta_1^g/2) + \Phi(\beta_1^g/2)]\,[\Phi(\beta_1^g/2) + 1 - \Phi(\beta_1^g/2)]} = 2\int_0^{\beta_1^g/2} f = 2\Phi(\beta_1^g/2) - 1.$$

We set r to $1/2$ and solve for β_1^g:

$$r(\beta_1^g) = 1/2 \iff 2\Phi(\beta_1^g/2) - 1 = 1/2 \iff \Phi(\beta_1^g/2) = 3/4 \iff \beta_1^g = 2\Phi^{-1}(3/4) \approx 1.35.$$

Because r is a strictly increasing function, it follows that $r(\beta_1^g) < 1/2$ for $\beta_1^g < 2\Phi^{-1}(3/4)$ and $r(\beta_1^g) > 1/2$ for $\beta_1^g > 2\Phi^{-1}(3/4)$. Next, because

$$b(\beta_1^g, c_{\text{bayes}}, 1/2) = 1 - \gamma(\beta_1^g, c_{\text{bayes}}, 1/2) = 1 - r(\beta_1^g),$$

we have that $b(\beta_1^g, c_{\text{bayes}}, 1/2) > 1/2$ for $\beta_1^g < 2\Phi^{-1}(3/4)$ and $b(\beta_1^g, c_{\text{bayes}}, 1/2) < 1/2$ for $\beta_1^g > 2\Phi^{-1}(3/4)$. Recall that the bias induced by sending the threshold to infinity (as stated in Proposition 6 and proven in Section A.7) is $1/2$, i.e.

$$b(\beta_1^g, \infty, 1/2) = 1/2.$$

We conclude that $b(\beta_1^g, c_{\text{bayes}}, 1/2) > b(\beta_1^g, \infty, 1/2)$ on $\beta_1^g \in [0, 2\Phi^{-1}(3/4))$; $b(\beta_1^g, c_{\text{bayes}}, 1/2) = b(\beta_1^g, \infty, 1/2)$ for $\beta_1^g = 2\Phi^{-1}(3/4)$; and $b(\beta_1^g, c_{\text{bayes}}, 1/2) < b(\beta_1^g, \infty, 1/2)$ on $\beta_1^g \in (2\Phi^{-1}(3/4), \infty)$.

A.10 Monotonicity in β_1^g

We show that γ is monotonically increasing in β_1^g for $\pi = 1/2$ and given threshold c. We begin by stating and proving two lemmas. The first lemma establishes an inequality that will serve as the basis for the proof.

Lemma 1. *The following inequality holds:*

$$\begin{aligned}[\Phi(\beta_0^g - c) + \Phi(\beta_0^g + \beta_1^g - c)] \cdot [\Phi(\beta_0^g + \beta_1^g - c) - \Phi(\beta_0^g - c) + \Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g)] \\ \geq [\Phi(\beta_0^g + \beta_1^g - c) - \Phi(\beta_0^g - c)]\,[\Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g)]\,. \quad (29)\end{aligned}$$

Proof: We take cases on the sign on β_1^g.

Case 1: $\beta_g^1 < 0$. Then $\beta_1^g + (\beta^g - c) < (\beta_0^g - c)$, implying $\Phi(\beta_0^g + \beta_1^g - c) < \Phi(\beta_0^g - c)$, or $[\Phi(\beta_0^g + \beta_1^g - c) - \Phi(\beta_0^g - c)] < 0$. Moreover, $[\Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g)]$ is positive. Therefore, the right-hand side of (29) is negative.

Turning our attention of the left-hand side of (29), we see that

$$\Phi(\beta_0^g + \beta_1^g - c) + \Phi(c - \beta_0^g - \beta_1^g) = 1 - \Phi(\beta_0^g + \beta_1^g - c) + \Phi(c - \beta_0^g - \beta_1^g) = 1. \qquad (30)$$

Additionally, $\Phi(\beta_0^g - c) < 1$ and $\Phi(c - \beta_0^g) > 0$. Combining these facts with (30), we find that

$$[\Phi(\beta_0^g + \beta_1^g - c) - \Phi(\beta_0^g - c) + \Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g)] > 0.$$

Finally, because $[\Phi(\beta_0^g - c) + \Phi(\beta_0^g + \beta_1^g - c)] > 0$, the entire left-hand side of (29) is positive. The inequality holds for $\beta_1^g < 0$.

Case 2: $\beta_g^1 \geq 0$. We will show that the first term on the LHS of (29) is greater than the first term on the RHS of (29), and likewise that the second term on the LHS is greater than the second term on the RHS, implying the truth of the inequality. Focusing on the first term, the positivity of $\Phi(\beta_0^g - c)$ implies that $\Phi(\beta_0^g - c) \geq -\Phi(\beta_0^g - c)$, and so

$$\Phi(\beta_0^g - c) + \Phi(\beta_0^g + \beta_1^g - c) \geq \Phi(\beta_0^g - \beta_1^g - c) - \Phi(\beta_0^g - c).$$

Next, focusing on the second term, $\beta_1^g \geq 0$ implies that

$$\beta_1^g + \beta_0^g - c \geq \beta_0^g - c \implies \Phi(\beta_1^g + \beta_0^g - c) - \Phi(\beta_0^g - c) \geq 0. \tag{31}$$

Adding $\Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g)$ to both sides of (31) yields

$$\Phi(\beta_1^g + \beta_0^g - c) - \Phi(\beta_0^g - c) + \Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g) \geq \Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g).$$

The inequality holds for $\beta_1^g \geq 0$. Combining the cases, the inequality holds for all $\beta_1^g \in \mathbb{R}$.

□

The second lemma establishes the derivatives of the functions $2 \cdot g$ and $4 \cdot h$ in β_1^g.

Lemma 2. *The derivatives in β_1^g of $2 \cdot g$ and $4 \cdot h$ are*

$$D_1[2g(\beta_1^g, c, 1/2)] = f(\beta_0^g + \beta_1^g - c), \tag{32}$$

$$\begin{aligned} D_1[4h(\beta_1^g, c, 1/2)] = {} & f(\beta_0^g + \beta_1^g - c)\,[\Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g)] \\ & - f(\beta_0^g + \beta_1^g - c)\,[\Phi(\beta_0^g - c) + \Phi(\beta_0^g + \beta_1^g - c)]. \end{aligned} \tag{33}$$

Proof: Apply FTC and product rule. $\square$

We are ready to prove the monotonicity of γ in β_1^g. Subtracting

$$[\Phi(\beta_0^g - c) + \Phi(\beta_0^g + \beta_1^g - c)]\,[\Phi(\beta_0^g + \beta_1^g - c) - \Phi(\beta_0^g - c)]$$

from both sides of (29) and multiplying by $f(\beta_0^g + \beta_1^g - c) > 0$ yields

$$\begin{aligned} f(\beta_0^g + \beta_1^g - c)&[\Phi(\beta_0^g - c) + \Phi\,(\beta_0^g + \beta_1^g - c)]\,[\Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g)] \\ &\geq f(\beta_0^g + \beta_1^g - c)\,[\Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g)][\Phi(\beta_0^g + \beta_1^g - c) - \Phi(\beta_0^g - c)] \\ &\quad - f(\beta_0^g + \beta_1^g - c)\,[\Phi(\beta_0^g - c) + \Phi(\beta_0^g + \beta_1^g - c)][\Phi(\beta_0^g + \beta_1^g - c) - \Phi(\beta_0^g - c)]. \end{aligned} \tag{34}$$

Next, recall that

$$2g(\beta_1^g, c, 1/2) = \Phi(\beta_0^g + \beta_1^g - c) - \Phi(\beta_0^g - c). \tag{35}$$

and

$$4h(\beta_1^g, c, 1/2) = [\Phi(\beta_0^g - c) + \Phi(\beta_0^g + \beta_1^g - c)]\,[\Phi(c - \beta_0^g) + \Phi(c - \beta_0^g - \beta_1^g)]. \tag{36}$$

Substituting (32, 33, 35, 36) into (34) produces

$$D_1[2g(\beta_1^g, c, 1/2)]4h(\beta_1^g, c, 1/2) \geq 2g(\beta_1^g, c, 1/2)D_1[4h(\beta_1^g, c, 1/2)],$$

or

$$D_1[2g(\beta_1^g, c, 1/2)]4h(\beta_1^g, c, 1/2) - 2g(\beta_1^g, c, 1/2)D_1[4h(\beta_1^g, c, 1/2)] \geq 0. \tag{37}$$

The quotient rule implies that

$$D_1\gamma(\beta_1^g, c, 1/2) = \frac{D_1[2g(\beta_1^g, c, 1/2)]4h(\beta_1^g, c, 1/2) - 2g(\beta_1^g, c, 1/2)D_1[4h(\beta_1^g, c, 1/2)]}{[4h(\beta_1^g, c, 1/2)]^2}. \quad (38)$$

We conclude by (37) and (38) that γ is monotonically increasing in β_1^g. Finally, $b(\beta_1^g, c, \pi) = 1 - \gamma(\beta_1^g, c, \pi)$ is monotonically decreasing in β_1^g.

A.11 Strict attenuation bias

We begin by computing the limit of γ in β_1^g given $\pi = 1/2$. First,

$$\begin{aligned}\lim_{\beta_1^g \to \infty} \gamma(\beta_1^g, c, 1/2) &= \frac{1 - \Phi(\beta_0^g - c)}{[1 + \Phi(\beta_0^g - c)]\,[\Phi(c - \beta_0^g)]} \\ &= \frac{\Phi(c - \beta_0^g)}{[1 + \Phi(\beta_0^g - c)]\,[\Phi(c - \beta_0^g)]} = \frac{1}{1 + \Phi(\beta_0^g - c)} < 1.\end{aligned}$$

Similarly,

$$\lim_{\beta_1^g \to -\infty} \gamma(\beta_1^g, c, 1/2) = \frac{-\Phi(\beta_0^g - c)}{[\Phi(\beta_0^g - c)]\,[\Phi(c - \beta_0^g) + 1]} = \frac{-1}{1 + \Phi(c - \beta_0^g)} > -1.$$

The function $\gamma(\beta_1^g, c, 1/2, \beta_0^g)$ is monotonically increasing in β_1^g (as stated in Proposition 4 and proven in section A.10). It follows that

$$-1 < -\frac{1}{1 + \Phi(c - \beta_0^g)} \leq \gamma(\beta_1^g, c, 1/2, \beta_0^g) \leq \frac{1}{1 - \Phi(\beta_0^g - c)} < 1$$

for all $\beta_1^g \in \mathbb{R}$. But β_0^g and c were chosen arbitrarily, and so

$$-1 < \gamma(\beta_1^g, c, 1/2, \beta_0^g) < 1$$

for all $(\beta_1^g, c, \beta_0^g) \in \mathbb{R}^3$. Finally, because $b(\beta_1^g, c, 1/2, \beta_0^g) = 1 - \gamma(\beta_1^g, c, 1/2, \beta_0^g)$, it follows that

$$0 < b(\beta_1^g, c, 1/2, \beta_0^g) < 2$$

for all $(\beta_1^g, c, \beta_0^g) \in \mathbb{R}^3$

A.12 Bias-variance decomposition in no-intercept model

We prove the bias-variance decomposition for the no-intercept version of (5). Define l (for "limit") by

$$l = \beta_m \left(\frac{\omega\pi}{\zeta(1-\pi) + \omega\pi} \right),$$

where

$$\omega = \bar{\Phi}(c - \beta_g) = \Phi(\beta_g - c); \quad \zeta = \bar{\Phi}(c) = \Phi(-c).$$

We have that

$$\hat{\beta}_m - l = \frac{\sum_{i=1}^n \hat{p}_i m_i}{\sum_{i=1}^n \hat{p}_i^2} - l = \frac{\sum_{i=1}^n \hat{p}_i m_i}{\sum_{i=1}^n \hat{p}_i^2} - \frac{l \sum_{i=1}^n \hat{p}_i^2}{\sum_{i=1}^n \hat{p}_i^2} = \frac{\sum_{i=1}^n \hat{p}_i (m_i - l\hat{p}_i)}{\sum_{i=1}^n \hat{p}_i^2}.$$

Therefore,

$$\sqrt{n}(\hat{\beta}_m - l) = \frac{(1/\sqrt{n}) \sum_{i=1}^n \hat{p}_i (m_i - l\hat{p}_i)}{(1/n) \sum_{i=1}^n \hat{p}_i^2}. \tag{39}$$

Next, we compute the expectation and variance of $\hat{p}_i(m_i - l\hat{p}_i)$. To do so, we first compute several simpler quantities:

1. Expectation of $\hat{p}_i$: $\mathbb{E}[\hat{p}_i] = \mathbb{P}(p_i\beta_g + \tau_i \geq c) = \mathbb{P}(\beta_g + \tau_i \geq c)\pi + \mathbb{P}(\tau_i \geq c)(1-\pi) = \pi\omega + (1-\pi)\zeta$.

2. Expectation of $\hat{p}_i p_i$: $\mathbb{E}\left[\hat{p}_i p_i\right] = \mathbb{E}\left[\hat{p}_i | p_i = 1\right] \mathbb{P}\left[p_i = 1\right] = \omega\pi$.

3. Expectation of $\hat{p}_i m_i$:

$$\mathbb{E}[\hat{p}_i m_i] = \mathbb{E}\left[\hat{p}_i(\beta_m p_i + \epsilon_i)\right] = \mathbb{E}\left[\beta_m \hat{p}_i p_i + \hat{p}_i \epsilon_i\right]$$
$$= \beta_m \mathbb{E}\left[\hat{p}_i p_i\right] + \mathbb{E}[\hat{p}_i]\mathbb{E}[\epsilon_i] = \beta_m \omega\pi + 0 = \beta_m \omega\pi.$$

4. Expectation of $\hat{p}_i m_i^2$:

$$\mathbb{E}\left[\hat{p}_i m_i^2\right] = \mathbb{E}\left[\hat{p}_i(\beta_m p_i + \epsilon_i)^2\right] = \mathbb{E}\left[\hat{p}_i\left(\beta_m^2 p_i^2 + 2\beta_m p_i \epsilon_i + \epsilon_i^2\right)\right]$$
$$= \mathbb{E}\left[\hat{p}_i p_i \beta_m^2 + 2\beta_m p_i \hat{p}_i \epsilon_i + \hat{p}_i \epsilon_i^2\right] = \beta_m^2 \mathbb{E}[\hat{p}_i p_i] + 2\beta_m \mathbb{E}[p_i \hat{p}_i]\mathbb{E}[\epsilon_i] + \mathbb{E}[\hat{p}_i]\mathbb{E}[\epsilon_i^2]$$
$$= \beta_m^2 \mathbb{E}[\hat{p}_i p_i] + \mathbb{E}[\hat{p}_i] = \beta_m^2 \omega\pi + \mathbb{E}[\hat{p}_i].$$

Now, we can compute the expectation and variance of $\hat{p}_i(m_i - l\hat{p}_i)$. First,

$$\mathbb{E}\left[\hat{p}_i(m_i - l\hat{p}_i)\right] = \mathbb{E}[\hat{p}_i m_i] - l\mathbb{E}[\hat{p}_i] = \beta_m \omega\pi - \left(\frac{\beta_m \omega\pi}{\zeta(1-\pi) + \omega\pi}\right)\left[\zeta(1-\pi) + \omega\pi\right] = 0. \quad (40)$$

Additionally,

$$\mathbb{V}\left[\hat{p}_i(m_i - l\hat{p}_i)\right] = \mathbb{E}\left[\hat{p}_i^2(m_i - l\hat{p}_i)^2\right] - \left(\mathbb{E}\left[\hat{p}_i(m_i - l\hat{p}_i)\right]\right)^2$$
$$= \mathbb{E}\left[\hat{p}_i m_i^2\right] - 2l\mathbb{E}[m_i \hat{p}_i] + l^2\mathbb{E}[\hat{p}_i] = \beta_m^2 \omega\pi + \mathbb{E}[\hat{p}_i] - 2l\beta_m \omega\pi + l^2 \mathbb{E}[\hat{p}_i]$$
$$= \beta_m \omega\pi(\beta_m - 2l) + \mathbb{E}[\hat{p}_i](1 + l^2). \quad (41)$$

Therefore, by CLT, (40), and (41),

$$(1/\sqrt{n})\sum_{i=1}^{n} \hat{p}_i(m_i - l\hat{p}_i) \xrightarrow{d} N\left(0, \beta_m \omega\pi(\beta_m - 2l) + \mathbb{E}[\hat{p}_i](1 + l^2)\right). \quad (42)$$

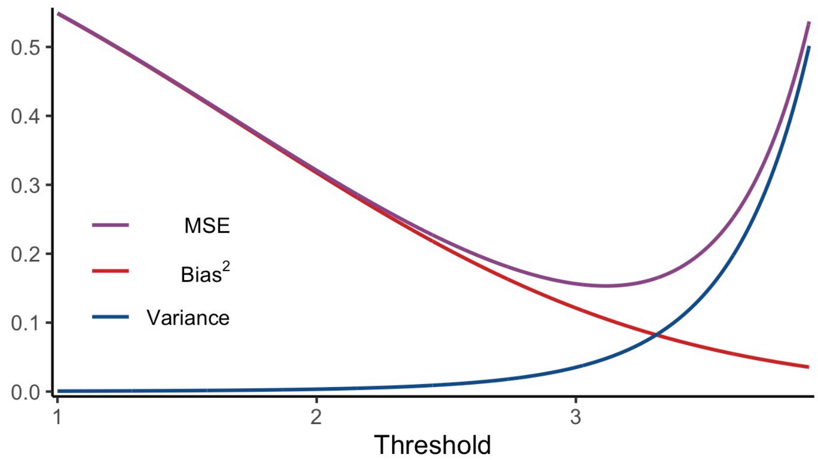

Figure 7: **Thresholding method bias-variance decomposition.** Bias decreases and variance increases as the threshold tends to infinity. $\beta_1^g = 1, \beta_1^m = 1$, and $\pi = 0.1$ in this plot.

Next, by weak LLN,

$$(1/n)\sum_{i=1}^{n} \hat{p}_i^2 = (1/n)\sum_{i=1}^{n} \hat{p}_i \xrightarrow{P} \mathbb{E}[\hat{p}_i]. \tag{43}$$

Finally, by (39), (42), (43), and Slutsky's Theorem,

$$\sqrt{n}(\hat{\beta}_m - l) \xrightarrow{d} N\left(0, \frac{\beta_m \omega \pi (\beta_m - 2l) + \mathbb{E}[\hat{p}_i](1 + l^2)}{\left(\mathbb{E}[\hat{p}_i]\right)^2}\right).$$

Thus, for large $n \in \mathbb{N}$, we have that

$$\mathbb{E}[\hat{\beta}_m] \approx l; \quad \mathbb{V}[\hat{\beta}_m] \approx \left[\beta_m \omega \pi (\beta_m - 2l) + \mathbb{E}[\hat{p}_i](1 + l^2)\right] / [n\mathbb{E}^2[\hat{p}_i]],$$

completing the bias-variance decomposition. Figure 7 plots the bias-variance decomposition as a function of the threshold.

B Estimation and inference in the GLM-EIV model

B.1 Estimation

We estimate the parameters of the GLM-EIV model using an EM algorithm.

E step

The E step entails computing the membership probability of each cell. Let $\theta^{(t)} = (\beta_m^{(t)}, \beta_g^{(t)}, \pi^{(t)})$ be the parameter estimate at the t-th iteration of the algorithm. For $k \in \{0, 1\}$, let $[\eta_i^m(k)]^{(t)}$ be the ith canonical parameter at the t-th iteration of the algorithm of the gene expression distribution that results from setting p_i to k, i.e. $[\eta_i^m(k)]^{(t)} \equiv h_m\left(\langle \tilde{x}_i(k), \beta_m^{(t)} \rangle + o_i^m\right)$. Similarly, let $[\eta_i^g(k)]^{(t)}$ be defined by $[\eta_i^g(k)]^{(t)} \equiv h_g\left(\langle \tilde{x}_i(k), \beta_g^{(t)} \rangle + o_i^g\right)$. Next, for $k \in \{0, 1\}$, define $\alpha_i^{(t)}(k)$ by

$$
\begin{aligned}
\alpha_i^{(t)}(k) &\equiv \mathbb{P}\left(M_i = m_i, G_i = g_i | P_i = k, \theta^{(t)}\right) \\
&= \mathbb{P}\left(M_i = m_i | P_i = k, \theta^{(t)}\right) \mathbb{P}\left(G_i = g_i | P_i = k, \theta^{(t)}\right) \text{ (because } G_i \perp\!\!\!\perp M_i | P_i) \\
&= f_m\left(m_i; [\eta_i^m(k)]^{(t)}\right) f_g\left(g_i; [\eta_i^g(k)]^{(t)}\right).
\end{aligned}
$$

Finally, let $\pi^{(t)}(1) \equiv \pi^{(t)} = \mathbb{P}\left(P_i = 1 | \theta^{(t)}\right)$ and $\pi^{(t)}(0) \equiv 1 - \pi^{(t)} = \mathbb{P}\left(P_i = 0 | \theta^{(t)}\right)$. The ith membership probability $T_i^{(t)}(1)$ is

$$
\begin{aligned}
T_i^{(t)}(1) &= \mathbb{P}(P_i = 1 | M_i = m_i, G_i = g_i, \theta^{(t)}) = \frac{\pi^{(t)}(1)\alpha_i^{(t)}(1)}{\sum_{k=0}^{1} \pi^{(t)}(k)\alpha_i^{(t)}(k)} \text{ (by Bayes rule)} \\
&= \frac{1}{\frac{\pi^{(t)}(0)\alpha_i(0)}{\pi^{(t)}(1)\alpha_i(1)} + 1} = \frac{1}{\exp\left(\log\left(\frac{\pi^{(t)}(0)\alpha_i(0)}{\pi^{(t)}(1)\alpha_i(1)}\right)\right) + 1} = \frac{1}{\exp\left(q_i^{(t)}\right) + 1},
\end{aligned} \tag{44}
$$

where we set

$$q_i^{(t)} := \log \left(\frac{\pi^{(t)}(0)\alpha_i^{(t)}(0)}{\pi^{(t)}(1)\alpha_i^{(t)}(1)} \right). \tag{45}$$

Next, we have that

$$\begin{aligned} q_i^{(t)} = \log \left[\pi^{(t)}(0)\right] + \log \left[f_m \left(m_i; [\eta_i^m(0)]^{(t)} \right) \right] + \log \left[f_g \left(g_i; [\eta_i^g(0)]^{(t)} \right) \right] \\ - \log \left[\pi^{(t)}(1)\right] - \log \left[f_m \left(m_i; [\eta_i^m(1)]^{(t)} \right) \right] - \log \left[f_g \left(g_i; [\eta_i^g(1)]^{(t)} \right) \right], \end{aligned}$$

We therefore conclude that $T_i^{(t)} = 1/\left(\exp\left(q_i^{(t)}\right) + 1\right)$, which is easily computable.

M step

The complete-data log-likelihood of the GLM-EIV model is

$$\mathcal{L}(\theta; m, g, p) = \sum_{i=1}^{n} \left[p_i \log(\pi) + (1-p_i)\log(1-\pi)\right] + \sum_{i=1}^{n} \log\left(f_m(m_i; \eta_i^m)\right) + \sum_{i=1}^{n} \log\left(f_g(g_i; \eta_i^g)\right). \tag{46}$$

Define $Q(\theta|\theta^{(t)}) = \mathbb{E}_{\left(P|M=m,G=g,\theta^{(t)}\right)} \left[\mathcal{L}(\theta; m, g, p)\right]$. We have that

$$\begin{aligned} Q(\theta|\theta^{(t)}) = \sum_{i=1}^{n} \left[T_i^{(t)}(1)\log(\pi) + T_i^{(t)}(0)\log(1-\pi) \right] \\ + \sum_{k=0}^{1}\sum_{i=1}^{n} T_i^{(t)}(k) \log\left[f_m(m_i; \eta_i^m(k))\right] + \sum_{k=0}^{1}\sum_{i=1}^{n} T_i^{(t)}(k) \log\left[f_g(g_i; \eta_i^{g,b}(k)) \right]. \end{aligned} \tag{47}$$

The three terms of (47) are functions of different parameters: the first is a function of π, the second is a function of β_m, and the third is a function of β_g. Therefore, to find the maximizer $\theta^{(t+1)}$ of (47), we maximize the three terms separately. Differentiating the first term with respect to π, we find that

$$\frac{\partial}{\partial \pi} \sum_{i=1}^{n} \left[T_i^{(t)}(1) \log(\pi) + T_i^{(t)}(0) \log(1-\pi) \right] = \frac{\sum_{i=1}^n T_i^{(t)}(1)}{\pi} - \frac{\sum_{i=1}^n T_i^{(t)}(0)}{1-\pi}.$$

Setting the derivative equal to 0 and solving for π,

$$\frac{\sum_{i=1}^n T_i^{(t)}(1)}{\pi} - \frac{\sum_{i=1}^n T_i^{(t)}(0)}{1-\pi} = 0 \iff \sum_{i=1}^n T_i^{(t)}(1) - \pi \sum_{i=1}^n T_i^{(t)}(1) = \pi \sum_{i=1}^n T_i(0)$$
$$\iff \sum_{i=1}^n T_i^{(t)}(1) - \pi \sum_{i=1}^n T_i^{(t)}(1) = \pi n - \pi \sum_{i=1}^n T_i(1) \iff \pi = \frac{\sum_{i=1}^n T_i^{(t)}(1)}{n}.$$

Thus, the maximizer $\pi^{(t+1)}$ of (47) in π is $\pi^{(t+1)} = (1/n) \sum_{i=1}^n T_i^{(t)}(1)$. Next, define $w^{(t)} = [T_1^{(t)}(0), \ldots, T_n^{(t)}(0), T_1^{(t)}(1), \ldots, T_n^{(t)}(1)]^T \in \mathbb{R}^{2n}$. We can view the second term of (47) as the log-likelihood of a GLM – call it $\text{GLM}_m^{(t)}$ – that has exponential family density f_m, link function r_m, responses $[m, m]^T$, offsets $[o^m, o^m]^T$, weights $w^{(t)}$, and design matrix $[\tilde{X}(0)^T, \tilde{X}(1)^T]^T$. Therefore, the maximizer $\beta_m^{(t+1)}$ of the second term of (47) is the maximizer of $\text{GLM}_m^{(t)}$, which we can compute using the iteratively reweighted least squares (IRLS) procedure, as implemented in R's GLM function. Similarly, the maximizer $\beta_g^{(t+1)}$ of the third term of (47) is the maximizer of the GLM with exponential family density f_g, link function r_g, responses $[g, g]^T$, offsets $[o^g, o^g]^T$, weights $w^{(t)}$, and design matrix $[\tilde{X}(0)^T, \tilde{X}(1)^T]^T$.

B.2 Inference

We derive the asymptotic observed information matrix of the GLM-EIV log likelihood, enabling us to perform inference on the parameters. First, we define some notation. For

$i \in \{1, \ldots, n\}$, $j \in \{0, 1\}$, and $\theta = (\pi, \beta_m, \beta_g)$, let $T_i^\theta(j)$ be defined by

$$T_i^\theta(j) = \mathbb{P}_\theta \left(P_i = j | M_i = m_i, G_i = g_i \right).$$

Let the $n \times n$ matrix $T^\theta(j)$ be given by $T^\theta(j) = \operatorname{diag}\left\{T_1^\theta(j), \ldots, T_n^\theta(j)\right\}$. Next, define the diagonal $n \times n$ matrices Δ^m, $[\Delta']^m$, V^m, and H^m by

$$\begin{cases} \Delta^m = \operatorname{diag}\{h_m'(l_1^m), \ldots, h_m'(l_n^m)\} \\ [\Delta']^m = \operatorname{diag}\{h_m''(l_1^m), \ldots, h_m''(l_n^m)\} \\ V^m = \operatorname{diag}\{\psi_m''(\eta_1^m), \ldots, \psi_m''(\eta_n^m)\} \\ H^m = \operatorname{diag}\{m_1 - \mu_1^m, \ldots, m_n - \mu_n^m\}. \end{cases}$$

Define the $n \times n$ matrices Δ^g, $[\Delta']^g$, V^g, and H^g analogously. These matrices are *unobserved*, as they depend on $\{p_1, \ldots, p_n\}$. Next, for $j \in \{0, 1\}$, let the diagonal $n \times n$ matrices $\Delta^m(j), [\Delta']^m(j), V^m(j)$, and $H^m(j)$ be given by

$$\begin{cases} \Delta^m(j) = \operatorname{diag}\{h_m'(l_1^m(j)), \ldots, h_m'(l_n^m(j))\} \\ [\Delta']^m(j) = \operatorname{diag}\{h_m''(l_1^m(j)), \ldots, h_m''(l_n^m(j))\} \\ V^m(j) = \operatorname{diag}\{\psi_m''(\eta_1^m(j)), \ldots, \psi_m''(\eta_n^m(j))\} \\ H^m(j) = \operatorname{diag}\{m_1 - \mu_1^m(j), \ldots, m_n - \mu_n^m(j)\}. \end{cases}$$

Define the matrices $\Delta^g(j)$, $[\Delta']^g(j)$, $V^g(j)$, and $H^g(j)$ analogously. Finally, define the vectors $s^m(j), w^m(j) \in \mathbb{R}^n$ by

$$\begin{cases} s^m(j) = [m_1 - \mu_1^m(j), \ldots, m_n - \mu_n^m(j)]^T \\ w^m(j) = [T_1(0)T_1(1)\Delta_1^m(j)H_1^m(j), \ldots, T_n(0)T_n(1)\Delta_n^m(j)H_n^m(j)]^T, \end{cases}$$

and let the vectors $s^g(j)$ and $w^g(j)$ be defined analogously. The quantities $\Delta^m(j), [\Delta']^m(j), V^m(j)$, $H^m(j)$, $s^m(j)$, $w^m(j)$, $\Delta^g(j), [\Delta']^g(j), V^g(j)$, $H^g(j)$, $s^g(j)$, and $w^g(j)$ are all *observed.*

The observed information matrix $J(\theta; m, g)$ evaluated at $\theta = (\pi, \beta_m, \beta_g)$ is the negative Hessian of the log likelihood (9) evaluated at θ, i.e. $J(\theta; m, g) = -\nabla^2 \mathcal{L}(\theta; m, g)$. This quantity, unfortunately, is hard to compute, as the log likelihood (9) is a complicated mixture. Louis (1982) showed that $J(\theta; m, g)$ is equivalent to the following quantity:

$$\begin{aligned} J(\theta; m, g) = &-\mathbb{E}\left[\nabla^2 \mathcal{L}(\theta; m, g, p) | G = g, M = m\right] \\ &+ \mathbb{E}\left[\nabla \mathcal{L}(\theta; m, g, p) | G = g, M = m\right] \mathbb{E}\left[\nabla \mathcal{L}(\theta; m, g, p) | G = g, M = m\right]^T \\ &- \mathbb{E}\left[\nabla \mathcal{L}(\theta; m, g, p) \nabla \mathcal{L}(\theta; m, g, p)^T | G = g, M = m\right]. \quad (48) \end{aligned}$$

The observed information matrix $J(\theta; m, g)$ has dimension $(2d+1) \times (2d+1)$. Recall that the complete-data log-likelihood (46) is the sum of three terms. The first term depends only on π, the second on β_m, and the third on β_g. Therefore, the observed information matrix can be viewed as block matrix consisting of nine submatrices (Figure 8; only six submatrices labelled). Submatrix I depends on π, submatrix II on β_m, submatrix III on β_g, submatrix IV on β_m and β_g, submatrix V on π and β_m, and submatrix VI on π and β_g. We only need to compute these six submatrices to compute the entire matrix, as the matrix is symmetric. The following sections derive formulas for submatrices I-VI. All expectations

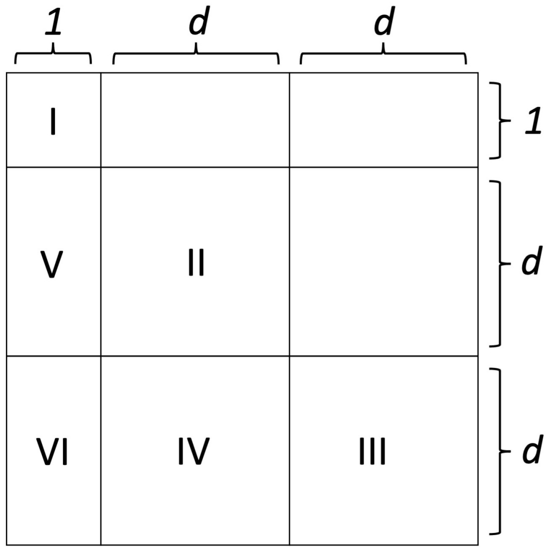

Figure 8: Block structure of the observed information matrix $J(\theta; m, g) = -\nabla^2 \mathcal{L}(\theta; m, g)$. The matrix is symmetric, and so we only need to compute submatrices I-VI to compute the entire matrix.

are understood to be *conditional* on m and g. The notation ∇_v and ∇_v^2 represent the gradient and Hessian, respectively, with respect to the vector v.

Submatrix I

Denote submatrix I by $J_\pi(\theta; m, g)$. The formula for $J_\pi(\theta; m, g)$ is

$$J_\pi(\theta; m, g) = -\mathbb{E}\left[\nabla_\pi^2 \mathcal{L}(\theta; m, g, p)\right] + \left(\mathbb{E}\left[\nabla_\pi \mathcal{L}(\theta; m, g, p)\right]\right)^2 - \mathbb{E}\left[\left(\nabla_\pi \mathcal{L}(\theta; m, g, p)\right)^2\right]. \quad (49)$$

We begin by calculating the first and second derivatives of the log-likelihood $\mathcal{L}$ with respect to π. The first derivative is

$$\nabla_\pi \mathcal{L}(\theta; m, g, p) = \frac{\partial}{\partial \pi}\left(\sum_{i=1}^n p_i \log(\pi) + \sum_{i=1}^n (1 - p_i) \log(1 - \pi)\right)$$
$$= \frac{\sum_{i=1}^n p_i}{\pi} - \frac{\sum_{i=1}^n (1 - p_i)}{1 - \pi} = \frac{\sum_{i=1}^n p_i}{\pi} - \frac{n - \sum_{i=1}^n p_i}{1 - \pi} = \left(\frac{1}{\pi} + \frac{1}{1 - \pi}\right)\sum_{i=1}^n p_i - \frac{n}{1 - \pi}. \quad (50)$$

The second derivative is

$$\nabla_\pi^2 \mathcal{L}(\theta; m, g, p) \quad = \quad \frac{\partial^2}{\partial^2 \pi}\left(\frac{\sum_{i=1}^n p_i}{\pi} - \frac{n - \sum_{i=1}^n p_i}{1-\pi}\right) \quad = \quad \frac{(\sum_{i=1}^n p_i) - n}{(1-\pi)^2} \quad - \quad \frac{\sum_{i=1}^n p_i}{\pi^2}.$$

We compute the expectation of the first term of (49):

$$\begin{aligned}
&\mathbb{E}\left[-\nabla_\pi^2 \mathcal{L}(\theta; m, g, p)\right] = -\mathbb{E}\left[\frac{(\sum_{i=1}^n p_i) - n}{(1-\pi)^2} - \frac{\sum_{i=1}^n p_i}{\pi^2}\right] \\
&= -\mathbb{E}\left\{\left[\frac{1}{(1-\pi)^2} - \frac{1}{\pi^2}\right]\sum_{i=1}^n p_i - \frac{n}{(1-\pi)^2}\right\} = -\left\{\left[\frac{1}{(1-\pi)^2} - \frac{1}{\pi^2}\right]\sum_{i=1}^n T_i^\theta(1) - \frac{n}{(1-\pi)^2}\right\} \\
&= \left[\frac{1}{\pi^2} - \frac{1}{(1-\pi)^2}\right]\sum_{i=1}^n T_i^\theta(1) + \frac{n}{(1-\pi)^2}. \quad (51)
\end{aligned}$$

Next, we compute the difference of the second two pieces of (49). To this end, define $a \equiv 1/(1-\pi) + 1/\pi$ and $b \equiv n/(1-\pi)$. We have that

$$\begin{aligned}
\mathbb{E}\left[\nabla_\pi \mathcal{L}(\theta; m, g, p)^2\right] &= \mathbb{E}\left[\left(a\sum_{i=1}^n p_i - b\right)^2\right] = \mathbb{E}\left[a^2\left(\sum_{i=1}^n p_i\right)^2 - 2ab\sum_{i=1}^n p_i + b^2\right] \\
&= a^2 \sum_{i=1}^n \sum_{j=1}^n \mathbb{E}[p_i p_j] - 2ab\sum_{i=1}^n \mathbb{E}[p_i] + b^2.
\end{aligned}$$

Next,

$$\left(\mathbb{E}\left[\nabla_\pi \mathcal{L}(\theta; m, g, x)\right]\right)^2 = \left(a\sum_{i=1}^n \mathbb{E}[p_i] - b\right)^2 = a^2\sum_{i=1}^n\sum_{j=1}^n \mathbb{E}[p_i]E[p_j] - 2ab\sum_{i=1}^n \mathbb{E}[p_i] + b^2.$$

Therefore,

$$\begin{aligned}
&(\mathbb{E}[\nabla_\pi \mathcal{L}(\theta; m, g, p)])^2 - \mathbb{E}\left[\nabla_\pi \mathcal{L}(\theta; m, g, p)^2\right] \\
&\quad = a^2\sum_{i=1}^n\sum_{j=1}^n \mathbb{E}[p_i]\mathbb{E}[p_j] - a^2\sum_{i=1}^n\sum_{j=1}^n \mathbb{E}[p_i p_j] = a^2\left(\sum_{i=1}^n \mathbb{E}[p_i]^2 - \mathbb{E}[p_i^2]\right)
\end{aligned}$$

$$= a^2 \left(\sum_{i=1}^{n} [T_i^\theta(1)]^2 - T_i^\theta(1) \right) = \left(\frac{1}{(1-\pi)} + \frac{1}{\pi} \right)^2 \left(\sum_{i=1}^{n} [T_i^\theta(1)]^2 - T_i^\theta(1) \right). \quad (52)$$

Stringing (49), (51) and (52) together, we obtain

$$J_\pi(\theta; m, g) = \left[\frac{1}{\pi^2} - \frac{1}{(1-\pi)^2} \right] \sum_{i=1}^{n} T_i^\theta(1) + \frac{n}{(1-\pi)^2} \\ + \left(\frac{1}{(1-\pi)} + \frac{1}{\pi} \right)^2 \left(\sum_{i=1}^{n} [T_i^\theta(1)]^2 - T_i^\theta(1) \right). \quad (53)$$

Submatrix II

Denote submatrix II by $J_{\beta^m}(\theta; m, g)$. The formula for $J_{\beta^m}(\theta; m, g)$ is

$$J_{\beta^m}(\theta; m, g) = -\mathbb{E}\left[\nabla^2_{\beta^m} \mathcal{L}(\theta; m, g, p)\right] \\ + \mathbb{E}\left[\nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)\right] \mathbb{E}\left[\nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)\right]^T - \mathbb{E}\left[\nabla_{\beta^m} \mathcal{L}(\theta; m, g, p) \nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)^T\right]. \quad (54)$$

Standard GLM results imply that $-\nabla^2_{\beta^m} \mathcal{L}(\theta; m, g, p) = \tilde{X}^T(\Delta^m V^m \Delta^m - [\Delta']^m H^m)\tilde{X}$ and $\nabla_{\beta^m} \mathcal{L}(\theta; m, g, p) = \tilde{X}^T \Delta^m s^m$. We compute the first term of (54). The (k, l)th entry of this matrix is

$$\left(\mathbb{E}\left[-\nabla^2_{\beta^m} \mathcal{L}(\theta; m, g, p)\right]\right)[k, l] = \mathbb{E}\left\{ \tilde{X}[, k]^T (\Delta^m V^m \Delta^m - [\Delta']^m H^m) \tilde{X}[, l] \right\} \\ = \sum_{i=1}^{n} \mathbb{E}\left\{ \tilde{x}_{i,k} (\Delta_i^m V_i^m \Delta_i^m - [\Delta']_i^m H_i^m) \tilde{x}_{i,l} \right\} \\ = \sum_{i=1}^{n} \tilde{x}_{i,k}(0) T_i^\theta(0) [\Delta_i^m(0) V_i^m(0) \Delta_i^m(0) - [\Delta']_i^m(0) H_i^m(0)] \tilde{x}_{i,l}(0) \\ + \sum_{i=1}^{n} \tilde{x}_{i,k}(1) T_i^\theta(1) [\Delta_i^m(1) V_i^m(1) \Delta_i^m(1) - [\Delta']_i^m(1) H_i^m(1)] \tilde{x}_{i,l}(1)$$

$$= \sum_{s=0}^{1} \tilde{X}(s)[,k]^T T^\theta(s) \left[\Delta^m(s) V^m(s) \Delta^m(s) - [\Delta']^m(s) H^m(s)\right] \tilde{X}(s)[,l].$$

We therefore have that

$$\mathbb{E}\left[-\nabla^2_{\beta^m} \mathcal{L}(\theta; m, g, p)\right] \;=\; \sum_{s=0}^{1} \tilde{X}(s)^T T^\theta(s) \left[\Delta^m(s) V^m(s) \Delta^m(s) - [\Delta']^m(s) H^m(s)\right] \tilde{X}(s). \tag{55}$$

Next, we compute the difference of the last two terms of (54). The (k,l)th entry is

$$\begin{aligned}
&\Bigg[\mathbb{E}\left[\nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)\right] \mathbb{E}\left[\nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)\right]^T \\
&\qquad\qquad - \mathbb{E}\left[\nabla_{\beta^m} \mathcal{L}(\theta; m, g, p) \nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)^T\right]\Bigg][k,l] \\
&= \left[\mathbb{E}\left[\tilde{X}^T \Delta^m s^m\right] \mathbb{E}\left[\tilde{X}^T \Delta^m s^m\right]^T\right][k,l] - \mathbb{E}\left[\tilde{X}^T \Delta^m s^m (s^m)^T \Delta^m \tilde{X}\right][k,l] \\
&= \mathbb{E}\left[\tilde{X}[,k]^T \Delta^m s^m\right] \mathbb{E}\left[\tilde{X}[,l]^T \Delta^m s^m\right] - \mathbb{E}\left[\tilde{X}[,k]^T \Delta^m s^m (s^m)^T \Delta^m \tilde{X}[,l]\right] \\
&= \mathbb{E}\left(\sum_{i=1}^n \tilde{x}_{ik} \Delta_i^m s_i^m\right) \mathbb{E}\left(\sum_{j=1}^n \tilde{x}_{jl} \Delta_j^m s_j^m\right) - \mathbb{E}\left(\sum_{i=1}^n \sum_{j=1}^n \tilde{x}_{ik} \Delta_i^m s_i^m s_j^m \Delta_j^m \tilde{x}_{jl}\right) \\
&= \sum_{i=1}^n \sum_{j=1}^n \mathbb{E}[\tilde{x}_{ik} \Delta_i^m s_i^m] \mathbb{E}[\tilde{x}_{jl} \Delta_j^m s_j^m] - \sum_{i=1}^n \sum_{j=1}^n \mathbb{E}[\tilde{x}_{ik} \Delta_i^m s_i^m s_j^m \Delta_j^m \tilde{x}_{jl}] \\
&= \sum_{i=1}^n \sum_{j=1}^n \mathbb{E}[\tilde{x}_{ik} \Delta_i^m s_i^m] \mathbb{E}\left[\tilde{x}_{jl} \Delta_j^m s_j^m\right] - \sum_{i \neq j} \mathbb{E}[\tilde{x}_{ik} \Delta_i^m s_i^m] \mathbb{E}[s_j^m \Delta_j^m \tilde{x}_{jl}] \\
&\qquad\qquad - \sum_{i=1}^n \mathbb{E}[\tilde{x}_{ik} \Delta_i^m s_i^m s_i^m \Delta_i^m \tilde{x}_{il}] \\
&= \sum_{i=1}^n \mathbb{E}[\tilde{x}_{ik} \Delta_i^m s_i^m] \mathbb{E}[\tilde{x}_{il} \Delta_i^m s_i^m] - \sum_{i=1}^n \mathbb{E}[\tilde{x}_{ik} (\Delta_i^m)^2 (H_i^m)^2 \tilde{x}_{il}] \\
&= \sum_{i=1}^n \left[\tilde{x}_{ik}(0) \Delta_i^m(0) T_i^\theta(0) H_i^m(0) + \tilde{x}_{ik}(1) \Delta_i^m(1) T_i^\theta(1) H_i^m(1)\right] \\
&\qquad \cdot \left[\tilde{x}_{il}(0) \Delta_i^m(0) T_i^\theta(0) H_i^m(0) + \tilde{x}_{il}(1) \Delta_i^m(1) T_i^\theta(1) H_i^m(1)\right] \\
&- \sum_{i=1}^n \left[\tilde{x}_{ik}(0) T_i^\theta(0) (\Delta_i^m(0))^2 (H_i^m(0))^2 \tilde{x}_{il}(0) + \tilde{x}_{ik}(1) T_i^\theta(1) (\Delta_i^m(1))^2 (H_i^m(1))^m \tilde{x}_{il}(1)\right]
\end{aligned}$$

$$
\begin{aligned}
&= \sum_{s=0}^{1}\sum_{t=0}^{1}\left[\sum_{i=1}^{n}\tilde{x}_{ik}(s)T_i^{\theta}(s)\Delta_i^m(s)H_i^m(t)T_i^{\theta}(t)\Delta_i^m(t)H_i^m(t)\tilde{x}_{il}(t)\right] \\
&\qquad - \sum_{s=0}^{1}\left[\sum_{i=1}^{n}\tilde{x}_{ik}(s)T_i^{\theta}(s)(\Delta_i^m(s))^2(H_i^m(s))^2\tilde{x}_{il}(s)\right] \\
&= \sum_{s=0}^{1}\sum_{t=0}^{1}\tilde{X}(s)[,k]^T T^{\theta}(s)\Delta^m(s)H^m(s)T^{\theta}(t)\Delta^m(t)H^m(t)\tilde{X}(k)[,l] \\
&\qquad\qquad - \sum_{s=0}^{1}X(s)[,k]^T T^{\theta}(s)(\Delta^m(s))^2(H^m(s))^2\tilde{X}(s)[,l].
\end{aligned}
$$

The sum of the last two terms on the right-hand side of (54) is therefore

$$
\begin{aligned}
&\mathbb{E}\left[\nabla_{\beta^m}\mathcal{L}(\theta;m,g,p)\right]\mathbb{E}\left[\nabla_{\beta^m}\mathcal{L}(\theta;m,g,p)\right]^T - \mathbb{E}\left[\nabla_{\beta^m}\mathcal{L}(\theta;m,g,p)\nabla_{\beta^m}\mathcal{L}(\theta;m,g,p)^T\right] \\
&\qquad = \sum_{s=0}^{1}\sum_{t=0}^{1}\tilde{X}(s)^T T^{\theta}(s)\Delta^m(s)H^m(s)T^{\theta}(t)\Delta^m(t)H^m(t)\tilde{X}(t) \\
&\qquad\qquad - \sum_{s=0}^{1}\tilde{X}(s)^T T^{\theta}(s)(\Delta^m(s))^2(H^m(s))^2\tilde{X}(s). \quad (56)
\end{aligned}
$$

Combining (54), (55), (56), we find that

$$
\begin{aligned}
J_{\beta^m}(\theta;m,g) &= \sum_{s=0}^{1}\tilde{X}(s)^T T^{\theta}(s)\left[\Delta^m(s)V^m(s)\Delta^m(s) - [\Delta']^m(s)H^m(s)\right]\tilde{X}(s) \\
&\quad + \sum_{s=0}^{1}\sum_{t=0}^{1}\tilde{X}(s)^T T^{\theta}(s)\Delta^m(s)H^m(s)T^{\theta}(t)\Delta^m(t)H^m(t)\tilde{X}(t) \\
&\qquad - \sum_{s=0}^{1}\tilde{X}(s)^T T^{\theta}(s)(\Delta^m(s))^2(H^m(s))^2\tilde{X}(s). \quad (57)
\end{aligned}
$$

Submatrix III

Denote submatrix III by $J_{\beta^g}(\theta;m,g)$. The formula for sub-matrix III is similar to that of sub-matrix II (57). Substituting g for m in this equation yields

$$
\begin{aligned}
J_{\beta^g}(\theta; m, g) = \sum_{s=0}^{1} \tilde{X}(s)^T T^\theta(s) \left[\Delta^g(s) V^g(s) \Delta^g(s) - [\Delta']^g(s) H^g(s)\right] \tilde{X}(s) \\
+ \sum_{s=0}^{1} \sum_{t=0}^{1} \tilde{X}(s)^T T^\theta(s) \Delta^g(s) H^g(s) T^\theta(t) \Delta^g(t) H^g(t) \tilde{X}(t) \\
- \sum_{s=0}^{1} \tilde{X}(s)^T T^\theta(s) (\Delta^g(s))^2 (H^g(s))^2 \tilde{X}(s). \quad (58)
\end{aligned}
$$

Submatrix IV

Denote sub-matrix IV by $J_{(\beta^g, \beta^m)}(\theta; m, g)$. The formula for $J_{(\beta^g, \beta^m)}(\theta; m, g)$ is

$$
\begin{aligned}
J_{(\beta^g, \beta^m)}(\theta; m, g) = \mathbb{E}\left[-\nabla_{\beta^g} \nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)\right] \\
+ \mathbb{E}\left[\nabla_{\beta^g} \mathcal{L}(\theta; m, g, p)\right] \mathbb{E}\left[\nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)\right]^T - \mathbb{E}\left[\nabla_{\beta^g} \mathcal{L}(\theta; m, g, p) \nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)^T\right].
\end{aligned} \quad (59)
$$

First, we have that

$$
\mathbb{E}\left[-\nabla_{\beta^g} \nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)\right] = 0, \quad (60)
$$

as differentiating $\mathcal{L}$ with with respect to β^g yields a vector that is a function of β^g, and differentiating this vector with respect to β^m yields 0. Next, recall from GLM theory that $\nabla_{\beta^g} \mathcal{L}(\theta; m, g, p) = \tilde{X}^T \Delta^g s^g$ and $\nabla_{\beta^m} \mathcal{L}(\theta; m, g, p) = \tilde{X}^T \Delta^m s^m$. The (k, l)th entry of the last two terms of (59) is

$$
\begin{aligned}
\Big[\mathbb{E}\left[\nabla_{\beta^g} \mathcal{L}(\theta; m, g, p)\right] \mathbb{E}\left[\nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)\right]^T \\
- \mathbb{E}\left[\nabla_{\beta^g} \mathcal{L}(\theta; m, g, p) \nabla_{\beta^m} \mathcal{L}(\theta; m, g, p)^T\right]\Big][k, l] \\
= \left[\mathbb{E}\left[\tilde{X}^T \Delta^g s^g\right] \mathbb{E}\left[\tilde{X}^T \Delta^m s^m\right]^T\right][k, l] - \mathbb{E}\left[\tilde{X}^T \Delta^g s^g (s^m)^T \Delta^m \tilde{X}\right][k, l] \\
= \mathbb{E}\left[\tilde{X}[, k]^T \Delta^g s^g\right] \mathbb{E}\left[\tilde{X}[, l]^T \Delta^m s^m\right] - \mathbb{E}\left[\tilde{X}[, k]^T \Delta^g s^g (s^m)^T \Delta^m \tilde{X}[, l]\right]
\end{aligned}
$$

$$
\begin{aligned}
&= \mathbb{E}\left(\sum_{i=1}^{n} \tilde{x}_{ik}\Delta_i^g s_i^g\right)\mathbb{E}\left(\sum_{j=1}^{n} \tilde{x}_{jl}\Delta_j^m s_j^m\right) - \mathbb{E}\left(\sum_{i=1}^{n}\sum_{j=1}^{n} \tilde{x}_{ik}\Delta_i^g s_i^g s_j^m \Delta_j^m \tilde{x}_{jl}\right) \\
&= \sum_{i=1}^{n}\sum_{j=1}^{n} \mathbb{E}[\tilde{x}_{ik}\Delta_i^g s_i^g]\mathbb{E}[\tilde{x}_{jl}\Delta_j^m s_j^m] - \sum_{i=1}^{n}\sum_{j=1}^{n} \mathbb{E}[\tilde{x}_{ik}\Delta_i^g s_i^g s_j^m \Delta_j^m \tilde{x}_{jl}] \\
&= \sum_{i=1}^{n}\sum_{j=1}^{n} \mathbb{E}[\tilde{x}_{ik}\Delta_i^g s_i^g]\mathbb{E}\left[\tilde{x}_{jl}\Delta_j^m s_j^m\right] - \sum_{i\neq j} \mathbb{E}[\tilde{x}_{ik}\Delta_i^g s_i^g]\mathbb{E}[\tilde{x}_{jl}\Delta_j^m s_j^m] \\
&\qquad - \sum_{i=1}^{n} \mathbb{E}[\tilde{x}_{ik}\Delta_i^g s_i^g s_i^m \Delta_i^m \tilde{x}_{il}] \\
&= \sum_{i=1}^{n} \mathbb{E}[\tilde{x}_{ik}\Delta_i^g H_i^g]\mathbb{E}[\tilde{x}_{il}\Delta_i^m H_i^m] - \sum_{i=1}^{n} \mathbb{E}[\tilde{x}_{ik} H_i^g \Delta_i^g \Delta_i^m H_i^m \tilde{x}_{il}] \\
&= \sum_{i=1}^{n} \left[\tilde{x}_{ik}(0)\Delta_i^g(0)T_i^\theta(0)H_i^g(0) + \tilde{x}_{ik}(1)\Delta_i^g(1)T_i^\theta(1)H_i^g(1)\right] \\
&\qquad \cdot \left[\tilde{x}_{il}(0)\Delta_i^m(0)T_i^\theta(0)H_i^m(0) + \tilde{x}_{il}(1)\Delta_i^m(1)T_i^\theta(1)H_i^m(1)\right] \\
&\qquad - \sum_{i=1}^{n}[\tilde{x}_{ik}(0)T_i^\theta(0)\Delta_i^g(0)H_i^g(0)\Delta_i^m(0)H_i^m(0)\tilde{x}_{il}(0) \\
&\qquad + \tilde{x}_{ik}(1)T_i^\theta(1)\Delta_i^g(1)H_i^g(1)\Delta_i^m(1)H_i^m(1)\tilde{x}_{il}(1)] \\
&= \sum_{s=0}^{1}\sum_{t=0}^{1}\left[\sum_{i=1}^{n} \tilde{x}_{ik}(s)T_i^\theta(s)\Delta_i^g(s)H_i^g(s)T_i^\theta(t)\Delta_i^m(t)H_i^m(t)\tilde{x}_{il}(t)\right] \\
&\qquad - \sum_{s=0}^{1}\left[\sum_{i=1}^{n} \tilde{x}_{ik}(s)T_i^\theta(s)\Delta_i^g(s)H_i^g(s)\Delta_i^m(s)H_i^m(s)\tilde{x}_{il}(s)\right] \\
&= \sum_{s=0}^{1}\sum_{t=0}^{1}\left[\tilde{X}(s)[,k]^T T^\theta(s)\Delta^g(s)H^g(s)T^\theta(t)\Delta^m(t)H^m(t)\tilde{X}(t)[,l]\right] \\
&\qquad - \sum_{s=0}^{1}\left[\tilde{X}[,k]^T T^\theta(s)\Delta^g(s)H^g(s)\Delta^m(s)H^m(s)\tilde{X}[,l](s)\right]. \qquad (61)
\end{aligned}
$$

Combining (59), (60), and (61) produces

$$
\begin{aligned}
J_{(\beta^g,\beta^m)}(\theta; m, g) = &\sum_{s=0}^{1}\sum_{t=0}^{1} \tilde{X}(s)^T T^\theta(s)\Delta^g(s)H^g(s)T^\theta(t)\Delta^m(t)H^m(t)\tilde{X}(t) \\
&- \sum_{s=0}^{1} \tilde{X}(s)^T T^\theta(s)\Delta^g(s)H^g(s)\Delta^m(s)H^m(s)\tilde{X}(s). \qquad (62)
\end{aligned}
$$

Submatrix V

Denote submatrix V by $J_{(\beta^m,\pi)}(\theta; m, g)$. The formula for $J_{(\beta^m,\pi)}(\theta; m, g)$ is

$$J_{(\beta^m,\pi)}(\theta; m, g) = \mathbb{E}\left[-\nabla_{\beta^m}\nabla_{\pi}\mathcal{L}(\theta; m, g, p)\right]$$
$$+ \mathbb{E}\left[\nabla_{\beta^m}\mathcal{L}(\theta; m, g, p)\right]\mathbb{E}\left[\nabla_{\pi}\mathcal{L}(\theta; m, g, p)\right]^T - \mathbb{E}\left[\nabla_{\beta^m}\mathcal{L}(\theta; m, g, p)\nabla_{\pi}\mathcal{L}(\theta; m, g, p)^T\right]. \quad (63)$$

We have that

$$\mathbb{E}\left[-\nabla_{\beta^m}\nabla_{\pi}\mathcal{L}(\theta; m, g, p)\right] = 0, \quad (64)$$

as β^m and π separate in the log likelihood. Next, set $a \equiv 1/\pi + 1/(1-\pi)$ and $b \equiv n/(1-\pi)$. Recall from GLM theory that $\nabla_{\beta^m}\mathcal{L}(\theta; m, g, p) = \tilde{X}^T\Delta^m s^m$ and from (50) that $a\sum_{i=1}^n p_i - b$. The kth entry of the last two terms of (63) is

$$\mathbb{E}\left[\nabla_{\pi}\mathcal{L}(\theta; m, g, p)\right]\mathbb{E}\left[\nabla_{\beta^m}\mathcal{L}(\theta; m, g, p)[k]\right] - \mathbb{E}\left[\nabla_{\pi}\mathcal{L}(\theta; m, g, p)\nabla_{\beta^m}\mathcal{L}(\theta; m, g, p)[k]\right]$$
$$= \left(\mathbb{E}\left[a\sum_{i=1}^n p_i - b\right]\right)\left(\mathbb{E}\left[\tilde{X}[,k]^T\Delta^m s^m\right]\right) - \mathbb{E}\left[\left(a\sum_{i=1}^n p_i - b\right)\tilde{X}[,k]^T\Delta^m s^m\right]$$
$$= \left(a\sum_{i=1}^n \mathbb{E}[p_i] - b\right)\left(\sum_{j=1}^n \mathbb{E}[\tilde{x}_{jk}\Delta_j^m s_j^m]\right) - \mathbb{E}\left[\left(a\sum_{i=1}^n p_i - b\right)\left(\sum_{j=1}^n \tilde{x}_{jk}\Delta_j^m s_j^m\right)\right]$$
$$= a\sum_{i=1}^n\sum_{j=1}^n \mathbb{E}[p_i]\mathbb{E}[\tilde{x}_{jk}\Delta_j^m s_j^m] - b\sum_{j=1}^n \mathbb{E}[\tilde{x}_{jk}\Delta_j^m s_j^m]$$
$$- \left[a\sum_{i=1}^n\sum_{j=1}^n \mathbb{E}[p_i\tilde{x}_{jk}\Delta_j^m s_j^m] - b\sum_{j=1}^n \mathbb{E}[\tilde{x}_{jk}\Delta_j^m s_j^m]\right]$$
$$= a\sum_{i=1}^n\sum_{j=1}^n \mathbb{E}[p_i]\mathbb{E}[\tilde{x}_{jk}\Delta_j^m s_j^m] - a\sum_{i\neq j} \mathbb{E}[p_i]\mathbb{E}[\tilde{x}_{jk}\Delta_j^m s_j^m] - a\sum_{i=1}^n \mathbb{E}[p_i\tilde{x}_{ik}\Delta_i^m s_i^m]$$
$$= a\sum_{i=1}^n \mathbb{E}[p_i]\mathbb{E}[\tilde{x}_{ik}\Delta_i^m s_i^m] - a\sum_{i=1}^n \mathbb{E}[p_i\tilde{x}_{ik}\Delta_i^m s_i^m]$$
$$= a\sum_{i=1}^n T_i^\theta(1)[T_i^\theta(0)\Delta_i^m(0)s_i^m(0)\tilde{x}_{ik}(0) + T_i^\theta(1)\Delta_i^m(1)s_i^m(1)\tilde{x}_{ik}(1)] - a\sum_{i=1}^n T_i^\theta(1)\Delta_i^m(1)s_i^m(1)\tilde{x}_{ik}(1)$$

$$
\begin{aligned}
&= a\sum_{i=1}^{n} T_i^{\theta}(0)T_i^{\theta}(1)\Delta_i^{m}(0)H_i^{m}(0)\tilde{x}_{ik}(0) \\
&\quad + a\sum_{i=1}^{n}\left([T_i^{\theta}(1)]^2\Delta_i^{m}(1)H_i^{m}(1) - T_i^{\theta}(1)\Delta_i^{m}(1)H_i^{m}(1)\right)\tilde{x}_{ik}(1) \\
&= a\left[\sum_{i=1}^{n} T_i^{\theta}(0)T_i^{\theta}(1)\Delta_i^{m}(0)H_i^{m}(0)\tilde{x}_{ik}(0) + \sum_{i=1}^{n} T_i^{\theta}(1)\Delta_i^{m}(1)H_i^{m}(1)[T_i^{\theta}(1)-1]\tilde{x}_{ik}(1)\right] \\
&= a\left[\sum_{i=1}^{n} T_i^{\theta}(0)T_i^{\theta}(1)\Delta_i^{m}(0)H_i^{m}(0)\tilde{x}_{ik}(0) - \sum_{i=1}^{n} T_i^{\theta}(0)T_i^{\theta}(1)\Delta_i^{m}(1)H_i^{m}(1)\tilde{x}_{ik}(1)\right] \\
&= a\left(\tilde{X}(0)[,k]^T w^{m}(0) - \tilde{X}(1)[,k]^T w^{m}(1)\right). \quad (65)
\end{aligned}
$$

Combining (63), (64), and (65), we conclude that

$$
J_{(\beta^m,\pi)}(\theta; m, g, p) = \left(\frac{1}{\pi} + \frac{1}{1-\pi}\right)\left(\tilde{X}(0)^T w^{m}(0) - \tilde{X}(1)^T w^{m}(1)\right). \quad (66)
$$

Submatrix VI

Denote submatrix VI by $J_{(\beta^g,\pi)}(\theta; m, g)$. Calculations similar to those for submatrix V show that

$$
J_{(\beta^g,\pi)}(\theta; m, g, p) = \left(\frac{1}{\pi} + \frac{1}{1-\pi}\right)\left(\tilde{X}(0)^T w^{g}(0) - \tilde{X}(1)^T w^{g}(1)\right). \quad (67)
$$

Combining submatrices

To summarize, the formulas for submatrices I-VI are as follows:

I

$$
\begin{aligned}
J_{\pi}(\theta; m, g) = \left[\frac{1}{\pi^2} - \frac{1}{(1-\pi)^2}\right]\sum_{i=1}^{n} T_i^{\theta}(1) + \frac{n}{(1-\pi)^2} \\
+ \left(\frac{1}{(1-\pi)} + \frac{1}{\pi}\right)^2 \left(\sum_{i=1}^{n}[T_i^{\theta}(1)]^2 - T_i^{\theta}(1)\right).
\end{aligned}
$$

II

$$
\begin{aligned}
J_{\beta^m}(\theta; m, g) = \sum_{s=0}^{1} \tilde{X}(s)^T T^\theta(s) \left[\Delta^m(s) V^m(s) \Delta^m(s) - [\Delta']^m(s) H^m(s)\right] \tilde{X}(s) \\
+ \sum_{s=0}^{1} \sum_{t=0}^{1} \tilde{X}(s)^T T^\theta(s) \Delta^m(s) H^m(s) T^\theta(t) \Delta^m(t) H^m(t) \tilde{X}(t) \\
- \sum_{s=0}^{1} \tilde{X}(s)^T T^\theta(s) (\Delta^m(s))^2 (H^m(s))^2 \tilde{X}(s).
\end{aligned}
$$

III

$$
\begin{aligned}
J_{\beta^g}(\theta; m, g) = \sum_{s=0}^{1} \tilde{X}(s)^T T^\theta(s) \left[\Delta^g(s) V^g(s) \Delta^g(s) - [\Delta']^g(s) H^g(s)\right] \tilde{X}(s) \\
+ \sum_{s=0}^{1} \sum_{t=0}^{1} \tilde{X}(s)^T T^\theta(s) \Delta^g(s) H^g(s) T^\theta(t) \Delta^g(t) H^g(t) \tilde{X}(t) \\
- \sum_{s=0}^{1} \tilde{X}(s)^T T^\theta(s) (\Delta^g(s))^2 (H^g(s))^2 \tilde{X}(s).
\end{aligned}
$$

IV

$$
\begin{aligned}
J_{(\beta^g, \beta^m)}(\theta; m, g) = \sum_{s=0}^{1} \sum_{t=0}^{1} \tilde{X}(s)^T T^\theta(s) \Delta^g(s) H^g(s) T^\theta(t) \Delta^m(t) H^m(t) \tilde{X}(t) \\
- \sum_{s=0}^{1} \tilde{X}(s)^T T^\theta(s) \Delta^g(s) H^g(s) \Delta^m(s) H^m(s) \tilde{X}(s).
\end{aligned}
$$

V

$$
J_{(\beta^m, \pi)}(\theta; m, g, p) = \left(\frac{1}{\pi} + \frac{1}{1-\pi}\right) \left(\tilde{X}(0)^T w^m(0) - \tilde{X}(1)^T w^m(1)\right).
$$

VI

$$
J_{(\beta^g, \pi)}(\theta; m, g, p) = \left(\frac{1}{\pi} + \frac{1}{1-\pi}\right) \left(\tilde{X}(0)^T w^g(0) - \tilde{X}(1)^T w^g(1)\right).
$$

We stitch these pieces together and transpose submatrices IV, V, and VI to produce the whole information matrix $J(\theta; m, g)$. Evaluating this matrix at the EM estimate θ^{EM} and inverting yields the asymptotic covariance matrix, which we can use to compute standard errors.

B.3 Implementation

To evaluate the observed information matrix, we need to compute the matrices $\Delta^m(j)$, $[\Delta']^m(j)$, $V^m(j)$, and $H^m(j)$ and the vectors $s^m(j)$ and $w^m(j)$ for $j \in \{0, 1\}$. We likewise need to compute the analogous gRNA quantities. The procedure that we propose for this purpose is general, but for concreteness, we describe how to implement this procedure in R by extending base family objects. We implicitly condition on p_i, z_i^m, and o_i^m.

An `R` family object contains several functions, including `linkinv`, `variance`, and `mu.eta`. `linkinv` is the inverse link function r_m^{-1}. `variance` takes as an argument the mean μ_i^m of the ith example and returns its variance $[\sigma_i^m]^2$. `mu.eta` is the derivative of the inverse link function $[r_m^{-1}]'$. We extend the `R` family object by adding two additional functions: `skewness` and `mu.eta.prime`. `skewness` returns the skewness γ_i^m of the distribution as a function of the mean μ_i, i.e.

$$\texttt{skewness}\,(\mu_i) = \mathbb{E}\left[\left(\frac{m_i - \mu_i^m}{\sigma_i^m}\right)^3\right] := \gamma_i^m.$$

Finally, `mu.eta.prime` is the second derivative of the inverse link function $[r_m^{-1}]''$. Algorithm 2 computes the matrices $\Delta^m(j)$, $[\Delta']^m(j)$, $V^m(j)$, and $H^m(j)$ and vector $s^m(j)$ for given β_m and given family object. (The vector $w^m(j)$ can be computed in terms of $\Delta^m(j)$ and $H^m(j)$.) We use $\sigma_i^m(j)$ (resp. $\gamma_i^m(j)$) to refer to the standard deviation (resp. skewness) of the gene expression distribution the ith cell when the perturbation p_i is set to j.

All steps of the algorithm are obvious except the calculation of $h'_m(l_i^m(j))$ (line 6), $h''(l_i^m(j))$ (line 9), and $V_i^m(j)$ (line 12). We omit the (j) notation for compactness. First, we prove the correctness of the expression for $h'_m(l_i^m)$. Recall the basic GLM identities

$$\psi''_m(\eta_i^m) = [\sigma_i^m]^2 \tag{68}$$

and, for all $t \in \mathbb{R}$,

$$r_m^{-1}(t) = \psi'_m(h_m(t)). \tag{69}$$

Differentiating (69) in t, we find that

$$(r_m^{-1})'(t) = \psi''_m(h_m(t))h'_m(t) \iff h'_m(t) = \frac{(r_m^{-1})'(t)}{\psi''_m(h_m(t))}. \tag{70}$$

Finally, plugging in l_i^m for t,

$$h'_m(l_i) = \frac{(r_m^{-1})'(l_i^m)}{\psi''(h_m(l_i^m))} = \frac{(r_m^{-1})'(l_i^m)}{\psi''_m(\eta_i^m)} = \text{ by (68) } \frac{(r_m^{-1})'(l_i^m)}{[\sigma_i^m]^2}.$$

Next, we prove the correctness for the expression for $h''_m(l_i^m)$. Recall the exponential family identity

$$\psi'''_m(\eta_i^m) = \gamma_i^m([\sigma_i^m]^2)^{3/2}. \tag{71}$$

Differentiating (70) in t, we obtain

$$(r_m^{-1})''(t) = \psi'''_m(h_m(t))[h'_m(t)]^2 + \psi''_m(h_m(t))h''_m(t) \iff h''_m(t) = \frac{(r_m^{-1})''(t) - \psi'''(h_m(t))[h'_m(t)]^2}{\psi''_m(h_m(t))}.$$

Plugging in l_i^m for t, we find that

$$h''_m(l_i^m) = \frac{(r_m^{-1})''(l_i^m) - \psi'''_m(\eta_i^m)[h'_m(l_i^m)]^2}{[\sigma_i^m]^2} = \text{ (by 71) } \frac{(r_m^{-1})''(l_i^m) - ([\sigma_i^m]^2)^{3/2}(\gamma_i^m)[h'_m(l_i^m)]^2}{[\sigma_i^m]^2}.$$

Finally, the expression for V_i^m follows from (68). We can apply a similar algorithm to compute the analogous matrices for the gRNA modality. Table 1 shows the `linkinv`, `variance`, `mu.eta`, `skewness`, and `mu.eta.prime` functions for several common family objects (which are defined by a distribution and link function).

Algorithm 2 Computing the matrices $\Delta^m(j)$, $[\Delta']^m(j)$, $V^m(j)$, $H^m(j)$, and $s^m(j)$ given given β_m.

Input: A coefficient vector β_m; data $[m_1, \ldots, m_n]$, $[o_1^m, \ldots, o_n^m]$, and $[z_1, \ldots, z_n]$; and a family object containing functions `linkinv`, `variance`, `mu.eta`, `mu.eta.prime`, and `skewness`.

for $j \in \{0, 1\}$ **do**

  for $i \in \{1, \ldots, n\}$ **do**

    $l_i^m(j) \leftarrow \langle \beta_m, \tilde{x}_i(j) \rangle + o_i^m$

    $\mu_i^m(j) \leftarrow \texttt{linkinv}(l_i^m(j))$

    $[\sigma_i^m(j)]^2 \leftarrow \texttt{variance}(\mu_i^m(j))$

    $h'_m(l_i^m(j)) \leftarrow \texttt{mu.eta}(l_i^m(j))/[\sigma_i^m(j)]^2$

    $\gamma_i^m(j) \leftarrow \texttt{skewness}(\mu_i^m(j))$

    $[r_m^{-1}]''(l_i^m(j)) \leftarrow \texttt{mu.eta.prime}(l_i^m(j))$

$$h''_m(l_i^m(j)) \leftarrow \frac{[r^{-1}]''(l_i^m(j)) - [([\sigma_i^m(j)]^2)^{3/2}][\gamma_i^m(j)][h'_m(l_i^m(j))]^2}{[\sigma_i^m(j)]^2}$$

▷ Assign quantities to matrices

    $\Delta_i^m(j) \leftarrow h'_m(l_i^m(j))$

    $[\Delta']_i^m(j) \leftarrow h''(l_i^m(j))$

    $V_i^m(j) \leftarrow [\sigma_i^m(j)]^2$

    $H_i^m(j) \leftarrow s_i^m(j) \leftarrow m_i - \mu_i^m(j)$

  end for

end for

Table 1: `linkinv`, `variance`, `mu.eta`, `skewness`, `mu.eta.prime` for common family objects (i.e., pairs of distributions and link functions).

	Gaussian response, identity link	Poisson response, log link	NB response ($\theta > 0$ fixed), log link
`linkinv`	x	$\exp(x)$	$\exp(x)$
`variance`	x	x	$x + x^2/\theta$
`mu.eta`	1	x	$\exp(x)$
`skewness`	0	$x^{-1/2}$	$\frac{2x+\theta}{\sqrt{\theta x}\sqrt{x+\theta}}$
`mu.eta.prime`	0	$\exp(x)$	$\exp(x)$

C Zero-inflated model

In this section we introduce the "zero-inflated" GLM-EIV model. The zero-inflated GLM-EIV model is appropriate to use when the unperturbed cells do not transcribe *any* gRNA molecules (i.e., when there are no background reads). Let $x_i = [1, z_i]^T \in \mathbb{R}^{d-1}$ be the vector of observed covariates, including an intercept term. (x_i is the same as $\tilde{x}_i$, but with the perturbation indicator p_i removed.) Let $\beta_{g,z} = [\beta_0^g, \gamma_g] \in \mathbb{R}^{d-1}$ be an unknown coefficient vector. ($\beta_{g,z}$ is the same as β_g, but with the perturbation effect β_1^g removed). Let the linear component $l_i^{g,z}$, mean $\mu_i^{g,z}$, and canonical parameter $\eta_i^{g,z}$ of gRNA count distribution of the ith cell be given by

$$l_i^{g,z} = \langle x_i, \beta_{g,z} \rangle + o_i^g; \quad r_g(\mu_i^{g,z}) = l_i^{g,z}; \quad \eta_i^{g,z} = ([\psi_g']^{-1} \circ r_g^{-1})(l_i^{g,z}) := h_g(l_i^{g,z}).$$

The density $f_{g,z}$ of gRNA counts in the zero-inflated model is as follows:

$$f_{g,z}(g_i; \eta_i^{g,z}, p_i) = [f_g(g_i; \eta_i^{g,z})]^{p_i} \mathbb{I}(g_i = 0)^{1-p_i}.$$

In other words, when the cell is *perturbed* (i.e., $p_i = 1$), the zero-inflated density $f_{g,z}$ coincides with the background-read density f_g; by contrast, when the cell is *unperturbed* (i.e., $p_i = 0$), the zero-inflated density $f_{g,z}$ is a point mass at zero. The gene expression density f_m and perturbation indicator density f_p are the same across the background read and zero-inflated models. We assume that the gene expression m_i and gRNA count g_i are conditionally independent given the perturbation indicator p_i. The joint density f_z of (m_i, p_i, z_i) is

$$f_z(m_i, g_i, p_i) = f_m(m_i|p_i) f_{g,z}(g_i|p_i) f_p(p_i) = \pi^{p_i}(1-\pi)^{1-p_i} f_m(m_i; \eta_i^m)[f_g(g_i; \eta_i^{g,z})]^{p_i} \mathbb{I}(g_i = 0)^{1-p_i}.$$

The complete-data log-likelihood $\mathcal{L}_z$ is

$$\begin{aligned} \mathcal{L}_z(\theta; m, g, p) = \sum_{i=1}^n \log\left[\pi^{p_i}(1-\pi)^{1-p_i}\right] + \sum_{i=1}^n \log\left[f_m(m_i; \eta_i^m)\right] \\ + \sum_{i=1}^n p_i \log\left[f_g(g_i; \eta_i^{g,z})\right] + \sum_{i=1}^n (1-p_i) \log\left[\mathbb{I}(g_i = 0)\right], \end{aligned}$$

where $\theta = [\pi, \beta_m, \beta_{g,z}]$ is the vector of unknown parameters. Integrating over the unobserved variable p_i, the marginal f_z of (m_i, g_i) is

$$f_z(m_i, g_i; \theta) = (1-\pi) f_m(m_i; \eta_i^m(0)) \mathbb{I}(g_i = 0) + \pi f_m(m_i; \eta_i^m(1)) f_g(g_i; \eta_i^{g,z}).$$

Finally, the log-likelihood is

$$\mathcal{L}_z(\theta; m_i, g_i) = \sum_{i=1}^n \log\left[(1-\pi) f_m(m_i; \eta_i^m(0)) \mathbb{I}(g_i = 0) + \pi f_m(m_i; \eta_i^m(1)) f_g(g_i; \eta_i^{g,z})\right].$$

C.1 Estimation

To estimate the parameters of the zero-inflated GLM-EIV model, we use an EM algorithm similar to Algorithm 1 but with two changes. First, we use a different formula for the ith membership probability at the t-th step of the algorithm $T_i^{(t)}(1)$. (We use $T_i^{(t)}(1)$ to denote the ith membership probability in *both* the background read and zero inflated cases; the difference should be clear from context.) Let $\theta^{(t)} = (\pi^{(t)}, \beta_m^{(t)}, \beta_{g,z}^{(t)})$ be the parameter estimate at the t-th iteration of the algorithm. Arguing in a manner similar to the background read case, we have that

$$T_i^{(t)}(1) = \frac{1}{\exp(q_i^{(t,z)}) + 1},$$

where

$$q_i^{(t,z)} = \log\left(\frac{(1-\pi^{(t)})\mathbb{P}(M_i = m_i | P_i = 0, \theta^{(t)})\mathbb{P}(G_i = g_i | P_i = 0, \theta^{(t)})}{(\pi^{(t)})\mathbb{P}(M_i = m_i | P_i = 1, \theta^{(t)})\mathbb{P}(G_i = g_i | P_i = 1, \theta^{(t)})}\right).$$

The expression for $q_i^{(t,z)}$ is

$$\begin{aligned} q_i^{(t,z)} = \log\left[1 - \pi^{(t)}\right] + \log\left[f_m\left(m_i; [\eta_i^m(0)]^{(t)}\right)\right] + \log\left[\mathbb{I}(g_i = 0)\right] \\ - \log\left[\pi^{(t)}\right] - \log\left[f_m\left(m_i; [\eta_i^m(1)]^{(t)}\right)\right] - \log\left[f_g\left(g_i; [\eta_i^{g,z}]^{(t)}\right)\right], \end{aligned}$$

where $[\eta_i^{g,z}]^{(t)} = h_g(\langle x_i, \beta_{g,z}^{(t)} \rangle + o_i^g)$. Notice that if $g_i \geq 1$, then $T_i^{(t)}(1) = 1$. This comports with our intuition that a nonzero gRNA count indicates the presence of a perturbation.

Next, we consider the M step of the EM algorithm, which is similar to the background read case. Define $Q_z(\theta|\theta^{(t)}) = \mathbb{E}_{(P|M=m,G=g,\theta^{(t)})}\left[\mathcal{L}_z(\theta; m, g, p)\right].$ We have that

$$\begin{aligned} Q_z(\theta|\theta^{(t)}) = \sum_{i=1}^n \left[T_i^{(t)}(1)\log(\pi) + T_i^{(t)}(0)\log(1-\pi)\right] + \sum_{i=1}^n \sum_{j=0}^1 T_i^{(t)}(j) \log\left[f_m(m_i; \eta_i^m(j))\right] \\ + \sum_{i=1}^n T_i^{(t)}(1)\left[\log(f_g(g_i; \eta_i^{g,z}))\right] + C. \quad (72) \end{aligned}$$

The three terms of (72) are functions of π, β_m, and $\beta_{g,z}$, respectively. The maximizer $\pi^{(t)}$

and $\beta_m^{(t+1)}$ of the first and second term are the same as in the background read case. The maximizer $\beta_{g,z}^{(t+1)}$ of the third term is the maximizer of the GLM with exponential family density f_g, link function r_g, responses g, weights $T^{(t)}(1)$, design matrix X, offsets o^g.

C.2 Inference

Next, we derive the asymptotic observed information matrix for the zero-inflated model, allowing us to perform inference. Again, let $T^\theta(1) := \text{diag}\{T_1^\theta(1), \ldots, T_n^\theta(1)\}$, but note that $T_i^\theta(1) = \mathbb{P}(P_i = 1 | G_i = g_i, M_i = m_i, \theta)$ is computed differently than in the background read case. Define the $n \times n$ matrices $\Delta^{(g,z)}, [\Delta']^{(g,z)}, V^{(g,z)}$, and $H^{(g,z)}$ by

$$\begin{cases} \Delta^{(g,z)} = \text{diag}\{h_g'(l_1^{g,z}), \ldots, h_g'(l_n^{g,z})\} \\ [\Delta']^{(g,z)} = \text{diag}\{h_g''(l_1^{g,z}), \ldots, h_g''(l_n^{g,z})\} \\ V^{(g,z)} = \text{diag}\{\psi_g(\eta_1^{g,z}), \ldots, \psi_g(\eta_n^{g,z})\} \\ H^{(g,z)} = \text{diag}\{m_1 - \mu_1^{g,z}, \ldots, m_n - \mu_n^{g,z}\}. \end{cases}$$

Also, define the $\mathbb{R}^n$ vectors $s^{(g,z)}$ and $w^{(g,z)}$ by

$$s^{(g,z)} = [g_1 - \mu_1^{g,z}, \ldots, g_n - \mu_n^{g,z}]^T,$$

and

$$w^{(g,z)} = [T_1^\theta(0)T_1^\theta(1)\Delta_1^{(g,z)}H_1^{(g,z)}, \ldots, T_n^\theta(0)T_n^\theta(1)\Delta_n^{(g,z)}H_n^{(g,z)}].$$

These quantities are computable, as they do not depend on the unobserved variables $p_1, \ldots, p_n$. Finally, let the unobserved, $n \times n$ matrix P be defined by $P = \text{diag}\{p_1, \ldots, p_n\}$.

The observed information matrix $J_z(\theta; m, g)$ is given by $J_z(\theta; m, g) = -\nabla^2 \mathcal{L}_z(\theta; m, g)$.

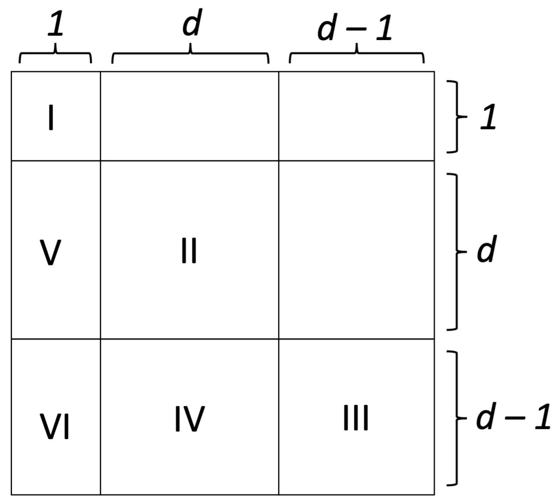

Figure 9: Block structure of the observed information matrix $J_z(\theta; m, g) = -\nabla^2 \mathcal{L}_z(\theta; m, g)$ for the zero-inflated model. Submatrices I, II, and VI are the same as in the background read model; therefore, we only need to compute submatrices III, VI, and V.

Louis's theorem implies that

$$J_z(\theta; m, g) = -\mathbb{E}\left[\nabla^2 \mathcal{L}_z(\theta; m, g, p) | G = g, M = m\right]$$
$$+ \mathbb{E}\left[\nabla \mathcal{L}_z(\theta; m, g, p) | G = g, M = m\right] \mathbb{E}\left[\nabla \mathcal{L}_z(\theta; m, g, p) | G = g, M = m\right]^T$$
$$- \mathbb{E}\left[\nabla \mathcal{L}_z(\theta; m, g, p) \nabla \mathcal{L}_z(\theta; m, g, p)^T | G = g, M = m\right].$$

The matrix $J_z(\theta; m, g)$ has dimension $d \times d$ and consists of nine submatrices (Figure 9). Three of these submatrices (i.e., I, II, and V) are the same as the corresponding submatrices in the background read case. We therefore must compute the remaining submatrices (i.e., III, IV, and VI) to compute the entire matrix $J_z(\theta; m, g)$. Again, in the following, all expectations are understood to be conditional on m and g.

Submatrix III (zero-inflated)

Denote submatrix III by $J_{\beta_{(g,z)}}(\theta; m, g)$ The formula for $J_{\beta_{(g,z)}}(\theta; m, g)$ is

$$J_{\beta_{(g,z)}}(\theta;m,g) = -\mathbb{E}\left[\nabla^2_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p)\right] + \mathbb{E}\left[\nabla_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p)\right]\mathbb{E}\left[\nabla_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p)\right]^T$$
$$- \mathbb{E}\left[\nabla_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p)\nabla_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p)^T\right]. \quad (73)$$

GLM theory indicates that $-\nabla^2_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p) = X^T P(\Delta^{(g,z)}V^{(g,z)}\Delta^{(g,z)} - (\Delta')^{(g,z)}H^{(g,z)})X$ and $\nabla_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p) = X^T P\Delta^{(g,z)}s^{(g,z)}$. We begin by computing the first term of (73). The only random matrix among X, P, $\Delta^{(g,z)}$, $V^{(g,z)}$, $(\Delta')^{(g,z)}$, and $H^{(g,z)}$ is P. Therefore, by the linearity of expectation,

$$-\mathbb{E}\left[\nabla^2_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p)\right] = \mathbb{E}\left[X^T P(\Delta^{(g,z)}V^{(g,z)}\Delta^{(g,z)} - (\Delta')^{(g,z)}H^{(g,z)}\right]$$
$$= X^T T^{\theta}(1)(\Delta^{(g,z)}V^{(g,z)}\Delta^{(g,z)} - (\Delta')^{(g,z)}H^{(g,z)})X. \quad (74)$$

Next, we compute the difference of the last two terms of (73). The (k,l)th entry of this matrix is

$$\Bigg[\mathbb{E}\left[\nabla_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p)\right]\mathbb{E}\left[\nabla_{\beta(g,z)}\mathcal{L}_z(\theta;m,g,p)\right]^T$$
$$- \mathbb{E}\left[\nabla_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p)\nabla_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p)^T\right]\Bigg][k,l]$$
$$= \left[\mathbb{E}\left[X^T P\Delta^{(g,z)}s^{(g,z)}\right]\mathbb{E}\left[X^T P\Delta^{(g,z)}s^{(g,z)}\right]^T\right][k,l] - \mathbb{E}\left[X^T P\Delta s^{(g,z)}(s^{(g,z)})^T\Delta^{(g,z)}PX^T\right][k,l]$$
$$= \mathbb{E}\left[X[,k]^T P\Delta^{(g,z)}s^{(g,z)}\right]\mathbb{E}\left[X[,l]^T P\Delta^{(g,z)}s^{(g,z)}\right] - \mathbb{E}\left[X[,k]^T P\Delta^{(g,z)}s^{(g,z)}(s^{(g,z)})^T\Delta^{(g,z)}PX[,l]\right]$$
$$= \mathbb{E}\left(\sum_{i=1}^n x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}\right)\mathbb{E}\left(\sum_{j=1}^n x_{jl}P_j\Delta_j^{(g,z)}s_j^{(g,z)}\right)$$
$$- \mathbb{E}\left(\sum_{i=1}^n\sum_{j=1}^n x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}s_j^{(g,z)}\Delta_j^{(g,z)}P_j x_{jl}\right)$$
$$= \sum_{i=1}^n\sum_{j=1}^n \mathbb{E}[x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}]\mathbb{E}[x_{jl}P_j\Delta_j^{(g,z)}s_j^{(g,z)}] - \sum_{i=1}^n\sum_{j=1}^n \mathbb{E}[x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}s_j^{(g,z)}\Delta_j^{(g,z)}P_j x_{jl}]$$
$$= \sum_{i=1}^n\sum_{j=1}^n \mathbb{E}[x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}]\mathbb{E}\left[x_{jl}P_j\Delta_j^{(g,z)}s_j^{(g,z)}\right] - \sum_{i\neq j}\mathbb{E}[x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}]\mathbb{E}[s_j^{(g,z)}P_j\Delta_j^{(g,z)}x_{jl}]$$

$$
\begin{aligned}
&- \sum_{i=1}^{n} \mathbb{E}[x_{ik} P_i \Delta_i^{(g,z)} s_i^{(g,z)} s_i^{(g,z)} \Delta_i^{(g,z)} P_i x_{il}] \\
&= \sum_{i=1}^{n} \mathbb{E}[x_{ik} P_i \Delta_i^{(g,z)} H_i^{(g,z)}] \mathbb{E}[x_{il} P_i \Delta_i^{(g,z)} H_i^{(g,z)}] - \sum_{i=1}^{n} \mathbb{E}[x_{ik} P_i^2 (\Delta_i^{(g,z)})^2 (H_i^{(g,z)})^2 x_{il}] \\
&= \sum_{i=1}^{n} x_{ik} T_i^{\theta}(1)^2 (\Delta_i^{(g,z)})^2 (H_i^{(g,z)})^2 x_{il} - \sum_{i=1}^{n} x_{ik} T_i^{\theta}(1) (\Delta_i^{(g,z)})^2 (H_i^{(g,z)})^2 x_{il} \\
&= X[,k]^T T^{\theta}(1)^2 (\Delta^{(g,z)})^2 (H^{(g,z)})^2 X[,l] - X[,k]^T T^{\theta}(1) (\Delta^{(g,z)})^2 (H^{(g,z)})^2 X[,l]
\end{aligned}
$$

Therefore, we have that

$$
\begin{aligned}
&\mathbb{E}\left[\nabla_{\beta_{(g,z)}} \mathcal{L}_z(\theta; m, g, p)\right] \mathbb{E}\left[\nabla_{\beta_{(g,z)}} \mathcal{L}_z(\theta; m, g, p)\right]^T - \mathbb{E}\left[\nabla_{\beta_{(g,z)}} \mathcal{L}_z(\theta; m, g, p) \nabla_{\beta_{(g,z)}} \mathcal{L}_z(\theta; m, g, p)^T\right] \\
&= X^T T^{\theta}(1)^2 (\Delta^{(g,z)})^2 (H^{(g,z)})^2 X - X^T T^{\theta}(1) (\Delta^{(g,z)})^2 (H^{(g,z)})^2 X \\
&= -X^T T^{\theta}(1) \left(\Delta^{(g,z)}\right)^2 \left(H^{(g,z)}\right)^2 \left(I - T^{\theta}(1)\right) X. \quad (75)
\end{aligned}
$$

Combining (73), (74), and (75), we conclude that

$$
\begin{aligned}
J_{\beta_{(g,z)}} = (\theta; m, g) &= X^T T^{\theta}(1) (\Delta^{(g,z)} V^{(g,z)} \Delta^{(g,z)} - (\Delta')^{(g,z)} H^{(g,z)}) X \\
&- X^T T^{\theta}(1) \left(\Delta^{(g,z)}\right)^2 \left(H^{(g,z)}\right)^2 \left(I - T^{\theta}(1)\right) X. \quad (76)
\end{aligned}
$$

Submatrix IV (zero-inflated)

Denote submatrix IV by $J_{(\beta_{(g,z)}, \beta_m)}(\theta; m, g)$. The formula for submatrix IV is

$$
\begin{aligned}
&J_{(\beta_{(g,z)}, \beta_m)}(\theta; m, g) = -\mathbb{E}\left[\nabla_{\beta_{(g,z)}} \nabla_{\beta_m} \mathcal{L}_z(\theta; m, g, p)\right] \\
&+ \mathbb{E}\left[\nabla_{\beta_{(g,z)}} \mathcal{L}_z(\theta; m, g, p)\right] \mathbb{E}\left[\nabla_{\beta_m} \mathcal{L}_z(\theta; m, g, p)\right]^T - \mathbb{E}\left[\nabla_{\beta_{(g,z)}} \mathcal{L}_z(\theta; m, g, p) \nabla_{\beta_m} \mathcal{L}_z(\theta; m, g, p)]^T\right]. \quad (77)
\end{aligned}
$$

First, we have that

$$-\mathbb{E}\left[\nabla_{\beta_{(g,z)}}\nabla_{\beta_m}\mathcal{L}_z(\theta;m,g,p)\right]=0, \tag{78}$$

as the derivative in β_m of $\mathcal{L}_z(\theta;m,g,p)$ is a function of β_m, and the derivative in $\beta_{(g,z)}$ of this term is 0. Next, we compute the difference of the last two terms of (77). Entry (k,l) of this matrix is

$$\begin{aligned}
&[\mathbb{E}[\nabla_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p)]\mathbb{E}[\nabla_{\beta_m}\mathcal{L}_z(\theta;m,g,p)]^T \\
&\qquad -\mathbb{E}[\nabla_{\beta_{(g,z)}}\mathcal{L}_z(\theta;m,g,p)\nabla_{\beta_m}\mathcal{L}_z(\theta;m,g,p)^T]][k,l] \\
&=\left[\mathbb{E}\left[X^TP\Delta^{(g,z)}s^{(g,z)}\right]\mathbb{E}\left[\tilde{X}^T\Delta^m s^m\right]^T\right][k,l]-\mathbb{E}\left[X^TP\Delta^{(g,z)}s^{(g,z)}(s^m)^T\Delta^m\tilde{X}\right][k,l] \\
&=\left[\mathbb{E}\left[X[,k]^TP\Delta^{(g,z)}s^{(g,z)}\right]\mathbb{E}\left[\tilde{X}[,l]^T\Delta^m s^m\right]^T\right]-\mathbb{E}\left[X[,k]^TP\Delta^{(g,z)}s^{(g,z)}(s^m)^T\Delta^m\tilde{X}[,l]\right] \\
&=\mathbb{E}\left(\sum_{i=1}^n x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}\right)\mathbb{E}\left(\sum_{j=1}^n \tilde{x}_{jl}\Delta_j^m s_j^m\right)-\mathbb{E}\left(\sum_{i=1}^n\sum_{j=1}^n x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}\Delta_j^m s_j^m\tilde{x}_{jl}\right) \\
&=\sum_{i=1}^n\sum_{j=1}^n\mathbb{E}[x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}]\mathbb{E}[\Delta_j^m s_j^m\tilde{x}_{jl}]-\sum_{i=1}^n\sum_{j=1}^n\mathbb{E}[x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}\Delta_j^m s_j^m\tilde{x}_{jl}] \\
&=\sum_{i=1}^n\sum_{j=1}^n\mathbb{E}[x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}]\mathbb{E}[\Delta_j^m s_j^m\tilde{x}_{jl}]-\sum_{i\neq j}\mathbb{E}[x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}]\mathbb{E}[\Delta_j^m s_j^m\tilde{x}_{jl}] \\
&\qquad -\sum_{i=1}^n\mathbb{E}[x_{ik}P_i\Delta_i^{(g,z)}s_i^{(g,z)}\Delta_j^m s_j^m\tilde{x}_{jl}] \\
&=\sum_{i=1}^n\mathbb{E}[x_{ik}P_i\Delta_i^{(g,z)}H_i^{(g,z)}]\mathbb{E}[\tilde{x}_{il}\Delta_i^m H_i^m]-\sum_{i=1}^n\mathbb{E}[x_{ik}P_i\Delta_i^{(g,z)}H_i^{(g,z)}\Delta_i^m H_i^m\tilde{x}_{il}] \\
&=\sum_{i=1}^n\left[x_{ik}T_i^\theta(1)\Delta_i^{(g,z)}H_i^{(g,z)}\right]\cdot\left[\Delta_i^m(0)T_i^\theta(0)H_i^m(0)\tilde{x}_{il}(0)+\Delta_i^m(1)T_i^\theta(1)H_i^m(1)\tilde{x}_{il}(1)\right] \\
&\qquad -\sum_{i=1}^n\left[x_{ik}T_i^\theta(1)\Delta_i^{(g,z)}H_i^{(g,z)}\Delta_i^m(1)H_i^m(1)\tilde{x}_{il}(1)\right] \\
&=\sum_{s=0}^1\sum_{i=1}^n x_{ik}T_i^\theta(1)H_i^{(g,z)}\Delta_i^{(g,z)}T_i^\theta(s)\Delta_i^m(s)H^m(s)\tilde{x}_{il}(s) \\
&\qquad -\sum_{i=1}^n\left[x_{il}T_i^\theta(1)\Delta_i^{(g,z)}H_i^{(g,z)}\Delta_i^m(1)H_i^m(1)\tilde{x}_{ik}(1)\right]
\end{aligned}$$

$$= \sum_{s=0}^{1} X[,k]^T T^\theta(1) H^{(g,z)} \Delta^{(g,z)} T^\theta(s) \Delta^m(s) H^m(s) \tilde{X}(s)[,l]$$

$$- X[,k]^T \Delta^{(g,z)} H^{(g,z)} T^\theta(1) \Delta^m(1) H^m(1) \tilde{X}[,l]. \quad (79)$$

Combining (73), (74), and (75) yields

$$J_{(\beta_{(g,z)},\beta_m)}(\theta; m, g) = \left(\sum_{s=0}^{1} X^T T^\theta(1) H^{(g,z)} \Delta^{(g,z)} T^\theta(s) \Delta^m(s) H^m(s) \tilde{X}(s) \right)$$

$$- X^T \Delta^{(g,z)} H^{(g,z)} T^\theta(1) \Delta^m(1) H^m(1) \tilde{X}(1). \quad (80)$$

Submatrix VI (zero-inflated)

Denote submatrix VI by $J_{(\beta_{(g,z)},\pi)}(\theta; m, g)$. The formula for $J_{(\beta_{(g,z)},\pi)}(\theta; m, g)$ is

$$J_{(\beta_{(g,z)},\pi)}(\theta; m, g) = \mathbb{E}\left[-\nabla_{\beta_{(g,z)}} \nabla_\pi \mathcal{L}_z(\theta; m, g, p) \right] + \mathbb{E}\left[\nabla_{\beta_{(g,z)}} \mathcal{L}_z(\theta; m, g, p) \right] \mathbb{E}\left[\nabla_\pi \mathcal{L}_z(\theta; m, g, p) \right]$$

$$- \mathbb{E}\left[\nabla_{\beta_{(g,z)}} \mathcal{L}_z(\theta; m, g, p) \nabla_\pi \mathcal{L}_z(\theta; m, g, p) \right]. \quad (81)$$

Recall that $\nabla_{\beta_{(g,z)}} \mathcal{L}_z(\theta; m, g, p) = X^T P \Delta^{(g,z)} s^{(g,z)}$ and $\nabla_\pi \mathcal{L}_z(\theta; m, g, p) = a \left(\sum_{i=1}^n p_i \right) - b$, where $a = 1/\pi + 1/(1-\pi)$ and $b = n/(1-\pi)$. We have that

$$\mathbb{E}\left[-\nabla_{\beta_{(g,z)}} \nabla_\pi \mathcal{L}_z(\theta; m, g, p) \right] = 0, \quad (82)$$

as the derivative in π of $\mathcal{L}_z(\theta; m, g, p)$ is a function of π, and the derivative in $\beta_{(g,z)}$ of this term is 0. Next, we compute the difference of the second two terms of (81). The kth entry of this vector is

$$\mathbb{E}\left[\nabla_\pi \mathcal{L}_z(\theta; m, g, p) \right] \mathbb{E}\left[\nabla_{\beta_{(g,z)}} \mathcal{L}_z(\theta; m, g, x)[k] \right] - \mathbb{E}\left[\nabla_\pi \mathcal{L}(\theta; m, g, p) \nabla_{\beta_{(g,z)}} \mathcal{L}(\theta; m, g, p)[k] \right]$$

$$
\begin{aligned}
&= \left(\mathbb{E}\left[a\sum_{i=1}^{n} p_i - b\right]\right)\left(\mathbb{E}\left[X[,k]^T P\Delta^{(g,z)} s^{(g,z)}\right]\right) - \mathbb{E}\left[\left(a\sum_{i=1}^{n} p_i - b\right) X[,k]^T P\Delta^{(g,z)} s^{(g,z)}\right] \\
&= \left(a\sum_{i=1}^{n}\mathbb{E}[p_i] - b\right)\left(\sum_{j=1}^{n}\mathbb{E}[x_{jk}p_j\Delta_j^{(g,z)}s_j^{(g,z)}]\right) \\
&\quad - \mathbb{E}\left[\left(a\sum_{i=1}^{n} p_i - b\right)\left(\sum_{j=1}^{n}\tilde{x}_{jk}p_j\Delta_j^{(g,z)}s_j^{(g,z)}\right)\right] \\
&= a\sum_{i=1}^{n}\sum_{j=1}^{n}\mathbb{E}[p_i]\mathbb{E}[x_{jk}p_j\Delta_j^{(g,z)}s_j^{(g,z)}] - b\sum_{j=1}^{n}\mathbb{E}[x_{jk}p_j\Delta_j^{(g,z)}s_j^{(g,z)}] \\
&\quad - \left[a\sum_{i=1}^{n}\sum_{j=1}^{n}\mathbb{E}[p_i x_{jk}p_j\Delta_j^{(g,z)}s_j^{(g,z)}] - b\sum_{j=1}^{n}\mathbb{E}[x_{jk}p_j\Delta_j^{(g,z)}s_j^{(g,z)}]\right] \\
&= a\sum_{i=1}^{n}\sum_{j=1}^{n}\mathbb{E}[p_i]\mathbb{E}[x_{jk}p_j\Delta_j^{(g,z)}s_j^{(g,z)}] - a\sum_{i\neq j}\mathbb{E}[p_i]\mathbb{E}[x_{jk}p_j\Delta_j^{(g,z)}s_j^{(g,z)}] - a\sum_{i=1}^{n}\mathbb{E}[x_{ik}p_i^2\Delta_i^{(g,z)}s_i^{(g,z)}] \\
&= a\sum_{i=1}^{n}\mathbb{E}[p_i]\mathbb{E}[x_{ik}p_i\Delta_i^{(g,z)}s_i^{(g,z)}] - a\sum_{i=1}^{n}\mathbb{E}[x_{ik}p_i^2\Delta_i^{(g,z)}s_i^{(g,z)}] \\
&= a\sum_{i=1}^{n} T_i^{\theta}(1)x_{ik}T_i^{\theta}(1)\Delta_i^{(g,z)}s_i^{(g,z)} - a\sum_{i=1}^{n} x_{ik}T_i^{\theta}(1)\Delta_i^{(g,z)}s_i^{(g,z)} \\
&= a\sum_{i=1}^{n}\left(x_{ik}T_i^{\theta}(1)^2\Delta_i^{(g,z)}s_i^{(g,z)} - x_{ik}T_i^{\theta}(1)\Delta_i^{(g,z)}s_i^{(g,z)}\right) = a\sum_{i=1}^{n} x_{ik}T_i^{\theta}(1)\Delta_i^{(g,z)}s_i^{(g,z)}\left(T_i^{\theta}(1) - 1\right) \\
&= -a\sum_{i=1}^{n} x_{ik}T_i(0)T_i^{\theta}(1)\Delta_i^{(g,z)}H_i^{(g,z)} = -aX[,k]^T w^{(g,z)}. \qquad (83)
\end{aligned}
$$

Combining (81), (82), and (83), we conclude that

$$
J_{(\beta_{(g,z)},\pi)}(\theta; m, g) = -\left(\frac{1}{\pi} + \frac{1}{1-\pi}\right) X^T w^{(g,z)}. \qquad (84)
$$

D Statistical accelerations and computing

D.1 Statistical accelerations

We describe in detail the procedure for obtaining the pilot parameter estimates $(\pi^{\text{pilot}}, \beta_m^{\text{pilot}}, \beta_g^{\text{pilot}})$. This procedure consists of two subroutines, which we label Algorithm 3 and Algorithm

4. The first step (Algorithm 3) is to obtain good parameter estimates for $[\beta_0^m, \gamma_m]^T$ and $[\beta_0^g, \gamma_g]^T$ via regression. Recall that the underlying gene expression parameter vector β_m is $\beta_m = [\beta_0^m, \beta_1^m, \gamma_m]^T \in \mathbb{R}^d$, where β_0^m is the intercept, β_1^m is the effect of the perturbation, and γ_m^T is the effect of the technical factors. To produce estimates $[\beta_0^m]^{\text{pilot}}$ and $[\gamma_m^T]^{\text{pilot}}$, we regress the gene expressions m onto the technical factors X. The intuition for this procedure is as follows: the probability of perturbation π is very small. Therefore, the true log likelihood is approximately equal to the log likelihood that results from omitting p_i from the model:

$$\begin{aligned}\sum_{i=1}^n f_m(m_i; \eta_i^m) &= \underbrace{\sum_{i:p_i=1} f_m(m_i; h_m(\beta_0 + \beta_1 + \gamma^T z_i + o_i^m))}_{\text{few terms}} + \underbrace{\sum_{i:p_i=0} f_m(m_i; h_m(\beta_0 + \gamma^T z_i + o_i^m))}_{\text{many terms}} \\ &\approx \sum_{i=1}^n f_m(m_i; h_m(\beta_0 + \gamma^T z_i + o_i^m)).\end{aligned}$$

We similarly can obtain pilot estimates $[\beta_0^g]^{\text{pilot}}$ and $[\gamma_g^T]^{\text{pilot}}$ by regressing the gRNA counts g onto the technical factors X. We extract the fitted values (on the scale of the linear component) for use in a subsequent step: $\hat{f}_i^k = [\beta_0^k]^{\text{pilot}} + \langle [\gamma_k^T]^{\text{pilot}}, z_i \rangle + o_i^k$, for $k \in \{m, g\}$.

Algorithm 3 Computing $[\beta_0^m]^{\text{pilot}}$, $[\gamma_m^T]^{\text{pilot}}$, $[\beta_0^g]^{\text{pilot}}$, and $[\gamma_g^T]^{\text{pilot}}$.

Input: Data m, g, o^m, o^g, and X; gene expression distribution f_m and link function r_m; gRNA expression distribution f_g and link function r_g; number of EM starts B.

for $k \in \{m, g\}$ **do**
  Fit a GLM GLM_k with responses k, offsets o^k, design matrix X, distribution f_k, and link function r_k.
  Set $[\beta_0^k]^{\text{pilot}}$ and $[\gamma_k^T]^{\text{pilot}}$ to the fitted coefficients of GLM_k.
  for $i \in \{1, \dots, n\}$ **do**
    $\hat{f}_i^k \leftarrow [\beta_0^k]^{\text{pilot}} + \langle [\gamma_k^T]^{\text{pilot}}, z_i \rangle + o_i^k$ ▷ untransformed fitted values
  end for
end for
return $([\beta_0^m]^{\text{pilot}}, \hat{f}^m, [\gamma_m^T]^{\text{pilot}}, [\beta_0^g]^{\text{pilot}}, [\gamma_g^T]^{\text{pilot}}, \hat{f}^g)$

Next, we obtain estimates $[\beta_1^m]^{\text{pilot}}$, $[\beta_1^g]^{\text{pilot}}$, and π^{pilot} for β_1^m, β_1^g, and π by fitting a "reduced" GLM-EIV (Algorithm 4). The log likelihood of the no-intercept, univariate GLM with predictor p_i and offset $\hat{f}_i^m$ is approximately equal to the true log likelihood:

$$\sum_{i=1}^{n} f_m(m_i; \eta_i^m) = \sum_{i=1}^{n} f_m(m_i; h_m(\beta_0 + \beta_1 p_i + \gamma^T z_i + o_i^m)) \approx \sum_{i=1}^{n} f_m(m_i; h_m(\beta_1 p_i + \hat{f}_i^m)).$$

Algorithm 4 Computing π^{pilot}, $[\beta_1^m]^{\text{pilot}}$, $[\beta_1^m]^{\text{pilot}}$.

Input: Data m, g; fitted offsets $\hat{f}^m$, $\hat{f}^g$.

`bestLik` $\leftarrow -\infty$ $\quad\triangleright$ Reduced GLM-EIV
for $i \in \{1, \ldots, B\}$ **do**
  Randomly generate starting parameters $\pi^{\text{curr}}, [\beta_1^m]^{\text{curr}}, [\beta_1^g]^{\text{curr}}$.
  while Not converged **do**
    for $i \in \{1, \ldots, n\}$ **do** $\quad\triangleright$ E step
      $T_i(1) \leftarrow \mathbb{P}(P_i = 1 | M_i = m_i, G_i = g_i, \pi^{\text{curr}}, [\beta_1^g]^{\text{curr}}, [\beta_1^m]^{\text{curr}})$
      $T_i(0) \leftarrow 1 - T_i(1)$
    end for
    $\pi^{\text{curr}} \leftarrow (1/n) \sum_{i=1}^n T_i(1)$ $\quad\triangleright$ M step
    $w \leftarrow [T_1(0), T_2(0), \ldots, T_n(0), T_1(1), T_2(1), \ldots, T_n(1)]^T$
    for $k \in \{g, m\}$ **do**
      Fit no-intercept, univariate GLM GLM_k with predictors $[\underbrace{0, \ldots, 0}_{\text{n}}, \underbrace{1, \ldots, 1}_{\text{n}}]$, responses $[k, k]^T$, offsets $[\hat{f}^k, \hat{f}^k]^T$, and weights w.
      Set $[\beta_1^k]^{\text{curr}}$ to fitted coefficient of GLM_k.
    end for
    Compute log likelihood `currLik` using π^{curr},$[\beta_1^m]^{\text{curr}}$, and $[\beta_1^g]^{\text{curr}}$.
  end while
  if `currLik` $>$ `bestLik` **then**
    bestLik $\leftarrow$ currLik
    $\pi^{\text{pilot}} \leftarrow \pi^{\text{curr}}$; $[\beta_1^m]^{\text{pilot}} \leftarrow [\beta_1^m]^{\text{curr}}$; $[\beta_1^g]^{\text{pilot}} \leftarrow [\beta_1^g]^{\text{curr}}$
  end if
end for
return $(\pi^{\text{pilot}}, [\beta_1^m]^{\text{pilot}}, [\beta_1^g]^{\text{pilot}})$

Therefore, to estimate β_1^m, β_1^g, and π, we fit a GLM-EIV model with gene expressions m, gRNA counts g, gene offsets $\hat{f}^m := [\hat{f}_1^m, \ldots, \hat{f}_n^m]^T$, gRNA offsets $\hat{f}^g := [\hat{f}_1^g, \ldots, \hat{f}_n^g]^T$, and *no* intercept or covariate terms. Intuitively, we "encode" all information about technical factors, library sizes, and baseline expression levels into $\hat{f}^m$ and $\hat{f}^g$. We run the algorithm $B \approx 15$ times over randomly-selected starting values for β^m, β^g, and π and select the solution with greatest the log likelihood.

The M step of the reduced GLM-EIV algorithm requires fitting two no-intercept, univariate GLMs with offsets. We derive analytic formulas for the MLEs of these GLMs in the three most important cases: Gaussian response with identity link, Poisson response with log link, and negative binomial response with log link (see section D.2; the latter formula is asymptotically exact). Consequently, we do not need to run the relatively slow IRLS procedure to carry out the M step of the reduced GLM-EIV algorithm. Overall, the proposed method for obtaining the full set of pilot parameter estimates requires fitting only two GLMs (via IRLS).

D.2 Intercept-plus-offset models

A key step in the algorithm for computing the pilot parameter estimates (Algorithm 4) is to fit a weighted, no-intercept, univariate GLM with nonzero offset terms and a binary predictor variable. We derive an analytic formula for the MLE of this GLM for three important pairs of response distributions and link functions: Gaussian response with identity link, Poisson response with log link, and negative binomial response with log link. The GLM that we seek to estimate has responses $[m, m]^T$, predictors $[\underbrace{0, \ldots, 0}_{\text{n}}, \underbrace{1, \ldots, 1}_{\text{n}}]$, offsets $[\hat{f}^m, \hat{f}^m]$, and weights $w = [T_1(0), \ldots, T_n(0), T_1(1), \ldots, T_n(1)]^T$. Throughout, C denotes a universal constant. The log likelihood of this GLM is

$$\mathcal{L}(\beta_1; m) = \sum_{i=1}^{n} T_i(0) f_m(m_i; h_m(\beta_1 + \hat{f}_i^m)) + \sum_{i=1}^{n} T_i(1) f_m(m_i; h_m(\hat{f}_i^m))$$
$$= \sum_{i=1}^{n} T_i(1) f_m(m_i; h_m(\beta_1 + \hat{f}_i^m)) + C. \quad (85)$$

Thus, finding the MLE $\hat{\beta}_1$ is equivalent to estimating a GLM with intercept β_1, offsets $\hat{f}^m$, weights $T_i(1)$, and *no* covariate terms. We term such a GLM a *intercept-plus-offset* model. Below, we study intercept-plus-offset models in generality.

General formulation Let $\beta \in \mathbb{R}$ be an unknown constant. Let $o_1, \ldots, o_n \sim \mathcal{P}_1$, where $\mathcal{P}_1$ is a distribution. Let $Y_i|o_i, \ldots, Y_n|o_i$ be exponential family-distributed random variables with identity sufficient statistic. Suppose the mean μ_i of $Y_i|o_i$ is given by $r(\mu_i) = \beta + o_i$, where $r : \mathbb{R} \to \mathbb{R}$ is a strictly increasing, differentiable link function. We call this model the *intercept-plus-offset* model.

We derive the (weighted) log likelihood of this model. Let $w_1, \ldots, w_n \sim \mathcal{P}_2$ be weights, where $\mathcal{P}_2$ is a distribution bounded above by 1 and below by 0. (A special case, which corresponds to no weights, is $w_i = 1$ for all $i \in \{1, \ldots, n\}$.) Throughout, we assume that $y_i w_i$ and $\exp(o_i)w_i$ have finite first moment. Suppose the cumulant-generating function and carrying density of the exponential family distribution are $\psi : \mathbb{R} \to \mathbb{R}$ and $c : \mathbb{R} \to \mathbb{R}$, respectively. The canonical parameter η_i of the ith observation is

$$\eta_i = ([\psi']^{-1} \circ r^{-1})(\beta + o_i) := h(\beta + o_i), \tag{86}$$

and the density f of $Y_i|\eta_i$ is $f(y_i; \eta_i) = \exp\{y_i\eta_i - \psi(\eta_i) + c(y_i)\}$. The weighted log likelihood is

$$\mathcal{L}(\beta; y_i) = \sum_{i=1}^{n} w_i \log\left[f(y_i; \eta_i)\right] = C + \sum_{i=1}^{n} w_i(y_i\eta_i - \psi(\eta_i)). \tag{87}$$

Our goal is to find the weighted MLE $\hat{\beta}$ of β. We consider three important choices for the exponential family distribution and link function. In the first two cases – Gaussian distribution with identity link and Poisson distribution with log link – we find the *finite-sample* maximizer of (87); by contrast, in the third case – negative binomial distribution with log link – we find an *asymptotically exact* maximizer.

Gaussian First, consider a Gaussian response distribution and identity link function $r(\mu) = \mu$. The cumulant-generating function ψ is $\psi(\eta) = \eta^2/2$, and so, by (86),

$$h(t) = [\psi']^{-1}(r^{-1}(t)) = [\psi']^{-1}(t) = t.$$

Plugging $\eta_i = h(\beta + o_i) = \beta + o_i$ and $\psi(\eta_i) = (1/2)(\beta + o_i)^2$ into (87), we obtain

$$\mathcal{L}(\beta; y) = \sum_{i=1}^{n} w_i(y_i(\beta + o_i) - (\beta + o_i)^2/2).$$

The derivative of this expression in β is

$$\frac{\partial \mathcal{L}(\beta; y)}{\partial \beta} = \sum_{i=1}^{n} w_i(y_i - \beta - o_i) = \sum_{i=1}^{n} w_i(y_i - o_i) - \beta \sum_{i=1}^{n} w_i.$$

Setting this quantity to 0 and solving for β, we find that the MLE $\hat{\beta}^{\text{gauss}}$ is

$$\hat{\beta}^{\text{gauss}} = \frac{\sum_{i=1}^{n} w_i(y_i - o_i)}{\sum_{i=1}^{n} w_i}.$$

Poisson Next, consider a Poisson response distribution and log link function $r(\mu) = \log(\mu)$. The cumulant-generating function ψ is $\psi(\eta) = e^{\eta}$. Therefore, by (86),

$$h(t) = [\psi']^{-1}(r^{-1}(t)) = [\psi']^{-1}(\exp(t)) = \log(\exp(t)) = t.$$

Plugging $\eta_i = h(\beta + o_i) = \beta + o_i$ and $\psi(\eta_i) = \exp(\beta + o_i)$ into (87), we obtain

$$\mathcal{L}(\beta; y) = \sum_{i=1}^{n} w_i \left(y_i(\beta + o_i) - \exp(\beta + o_i) \right).$$

The derivative of this function in β is

$$\frac{\partial \mathcal{L}(\beta; y)}{\partial \beta} = \sum_{i=1}^{n} w_i y_i - w_i \exp(\beta + o_i) = \sum_{i=1}^{n} w_i y_i - \exp(\beta) \sum_{i=1}^{n} w_i \exp(o_i).$$

Setting to zero and solving for β, we find that the MLE $\hat{\beta}^{\text{pois}}$ is

$$\hat{\beta}^{\text{pois}} = \log \left(\frac{\sum_{i=1}^{n} w_i y_i}{\sum_{i=1}^{n} w_i e^{o_i}} \right). \tag{88}$$

Negative binomial Finally, we consider a negative binomial response distribution (with fixed size parameter $\theta > 0$) and log link function $r(\mu) = \log(\mu)$. The cumulant-generating function ψ is $\psi(\eta) = -\theta \log(1 - e^{\eta})$. The derivative ψ' of ψ is

$$\psi'(t) = \theta \left(\frac{e^t}{1 - e^t} \right) = \frac{\theta}{e^{-t} - 1}.$$

Define the function $\delta : \mathbb{R} \to \mathbb{R}$ by $\delta(t) = -\log\left(\theta/t + 1\right)$. We see that

$$\psi'(\delta(t)) = \frac{\theta}{\exp\left(\log(\theta/t + 1)\right) - 1} = t,$$

implying $\delta = [\psi']^{-1}$. By (86), we have that

$$h(t) = [\psi']^{-1}(r^{-1}(t)) = -\log\left(\frac{\theta}{\exp(t)} + 1 \right) = \log\left(\frac{\exp(t)}{\theta + \exp(t)} \right).$$

Therefore,

$$\eta_i = h(\beta+o_i) = \log\left(\frac{\exp(\beta+o_i)}{\theta+\exp(\beta+o_i)}\right) = \beta+o_i-\log\left(\theta+e^{\beta}e^{o_i}\right) = \beta-\log\left(\theta+e^{\beta}e^{o_i}\right)+C, \tag{89}$$

and

$$\begin{aligned}\psi(\eta_i) = -\theta\log\left(1-\frac{\exp(\beta+o_i)}{\theta+\exp(\beta+o_i)}\right) &= -\theta\log\left(\frac{\theta}{\theta+\exp(\beta+o_i)}\right)\\ &= -\theta\log(\theta)+\theta\log[\theta+\exp(\beta+o_i)] = \theta\log(\theta+e^{\beta}e^{o_i})+C.\end{aligned} \tag{90}$$

Plugging (89) and (90) into (87), the log-likelihood (up to a constant) is

$$\begin{aligned}\mathcal{L}(\beta;y) = \beta\sum_{i=1}^{n} w_i y_i - \sum_{i=1}^{n} w_i y_i \log(\theta+e^{\beta}e^{o_i}) - \theta\sum_{i=1}^{n} w_i\log(\theta+e^{\beta}e^{o_i})\\ = \beta\sum_{i=1}^{n} w_i y_i - \sum_{i=1}^{n}(y_i+\theta)w_i\log(\theta+e^{\beta}e^{o_i}).\end{aligned}$$

The derivative of $\mathcal{L}$ in β is

$$\frac{\partial\mathcal{L}(\beta;y)}{\partial\beta} = \sum_{i=1}^{n} w_i y_i - \sum_{i=1}^{n}\frac{w_i(y_i+\theta)e^{\beta}e^{o_i}}{\theta+e^{\beta}e^{o_i}}.$$

Setting the derivative to zero, the equation defining the MLE is

$$e^{\beta}\sum_{i=1}^{n}\frac{w_i e^{o_i}(y_i+\theta)}{e^{\beta}e^{o_i}+\theta} = \sum_{i=1}^{n} w_i y_i. \tag{91}$$

We cannot solve for β in (91) analytically. However, we can derive an asymptotically exact solution. By the law of total expectation,

$$\mathbb{E}\left[\frac{w_i e^{o_i}(y_i+\theta)}{e^{\beta+o_i}+\theta}\right] = \mathbb{E}\left[\mathbb{E}\left[\frac{w_i e^{o_i}(y_i+\theta)}{e^{\beta+o_i}+\theta}\middle|(o_i,w_i)\right]\right] = \mathbb{E}\left[\frac{w_i e^{o_i}(e^{\beta+o_i}+\theta)}{e^{\beta+o_i}+\theta}\right] = \mathbb{E}[w_i e^{o_i}];$$

the second equality holds because $\mathbb{E}[y_i|o_i] = \mu_i = e^{\beta+o_i}$. Dividing by n on both sides of (91) and rearranging,

$$\beta = \log\left(\frac{(1/n)\sum_{i=1}^n w_i e^{o_i}(y_i+\theta)/(e^\beta e^{o_i}+\theta)}{(1/n)\sum_{i=1}^n w_i y_i.}\right). \tag{92}$$

By weak LLN, the limit (in probability) of the MLE $\hat{\beta}^{\text{NB}}$ is

$$\hat{\beta}^{\text{NB}} \xrightarrow{P} \log\left(\frac{\mathbb{E}[w_i y_i]}{\mathbb{E}[w_i e^{o_i}]}\right). \tag{93}$$

But the Poisson MLE $\hat{\beta}^{\text{Pois}}$ (88) converges in probability to the same limit:

$$\hat{\beta}^{\text{pois}} = \log\left(\frac{(1/n)\sum_{i=1}^n w_i y_i}{(1/n)\sum_{i=1}^n w_i e^{o_i}}\right) \xrightarrow{P} \log\left(\frac{\mathbb{E}[w_i y_i]}{\mathbb{E}[w_i e^{o_i}]}\right).$$

Therefore, for large n, we can approximate $\hat{\beta}^{\text{NB}}$ by $\hat{\beta}^{\text{pois}}$.

Application to GLM-EIV The GLM that we seek to estimate (85) is an approximate intercept-plus-offset model: $T_1(1),\ldots,T_n(1)$ are the weights $w_1,\ldots,w_n$, and $\hat{f}_1^m,\ldots,\hat{f}_n^m$ are the offsets $o_1,\ldots,o_m$. Of course, $T_1(1),\ldots,T_1(n)$ are in general dependent random variables, as are $\hat{f}_1^m,\ldots,\hat{f}_n^m$. $T_i(1)$ depends on m_i and g_i, as well as the final parameter estimate $(\hat{\pi},\hat{\beta}_m,\hat{\beta}_g)$, which itself is a function of m and g; the situation is similar for the $\hat{f}_i^m$s. In practice, we find that the intercept-plus-offset model is very good approximation to the GLM (85), especially when the number of cells n is large. Additionally, we note that the GLM (85) is fitted as a subroutine of the algorithm for producing pilot parameter estimates (Algorithm 4). The quality of the pilot parameter estimates does not affect the validity of the estimation and inference procedures (Algorithm 1), barring issues related to convergence to local optima.

D.3 Computing

We describe in detail the at-scale GLM-EIV pipeline. First, we run a round of "precomputations" on all d_g genes and d_p perturbations. The precomputations involve regressing the gene expressions (or gRNA counts) onto the technical factors, thereby "factoring out" Algorithm 3. Next, we run differential expression analyses on the full set of gene-perturbation pairs; for a given pair, this amounts to obtaining the complete set of pilot parameters (by running a reduced GLM-EIV), fitting the GLM-EIV model (Algorithm 1), and performing inference. The three loops in Algorithm 5 are embarassingly parallel and therefore can be massively parallelized.

Algorithm 5 Applying GLM-EIV at scale.

$G \leftarrow \{\text{gene}_1, \ldots, \text{gene}_{d_g}\}; P \leftarrow \{\text{perturbation}_1, \ldots, \text{perturbation}_{d_p}\}$
for gene $\in G$ **do**
  Run precomputation (Algorithm 3) on gene; save $\hat{f}^m$, $[\beta_0^m]^{\text{pilot}}$ and $[\gamma_m^T]^{\text{pilot}}$.
end for
for perturbation $\in P$ **do**
  Run precomputation (Algorithm 3) on perturbation; save $\hat{f}^g$, $[\beta_0^g]^{\text{pilot}}$ and $[\gamma_g^T]^{\text{pilot}}$.
end for
for (gene, perturbation) $\in G \times P$ **do**
  Load $\hat{f}^m$, $\hat{f}^g$, $[\beta_0^m]^{\text{pilot}}$ $[\gamma_m^T]^{\text{pilot}}$, $[\beta_0^g]^{\text{pilot}}$ and $[\gamma_g^T]^{\text{pilot}}$.
  Compute $[\beta_1^m]^{\text{pilot}}, [\beta_1^g]^{\text{pilot}}, \pi^{\text{pilot}}$ by fitting a reduced GLM-EIV (Algorithm 4).
  Run GLM-EIV using the pilot parameters (Algorithm 1).
end for

E Additional simulation study

We ran an additional simulation study in which we modeled the gene and gRNA expressions using a Gaussian distribution with identity link. We generated data on $n = 150,000$ cells, fixing the target of inference β_1^m to -4 and the probability of perturbation π to 0.05. We included "sequencing batch" (modeled as a Bernoulli-distributed variable) and "sequencing depth" (modeled as a Poisson-distributed variable) as covariates in the model. We did not include sequencing depth as an offset because use of the identity link renders offsets meaningless. We varied β_1^g over a grid on the interval $[0, 7]$. We generated $n_{\text{sim}} = 1,000$ synthetic datasets for each value of β_1^g. We applied accelerated GLM-EIV and thresholded regression to the simulated data. We assessed these methods on the metrics of bias, mean squared error, confidence interval coverage rate, and confidence interval width. We found that accelerated GLM-EIV outperformed the thresholding method: the former method exhibited smaller bias, smaller mean squared error, higher confidence interval coverage rate, and smaller confidence interval width than the latter method (Figure 10).

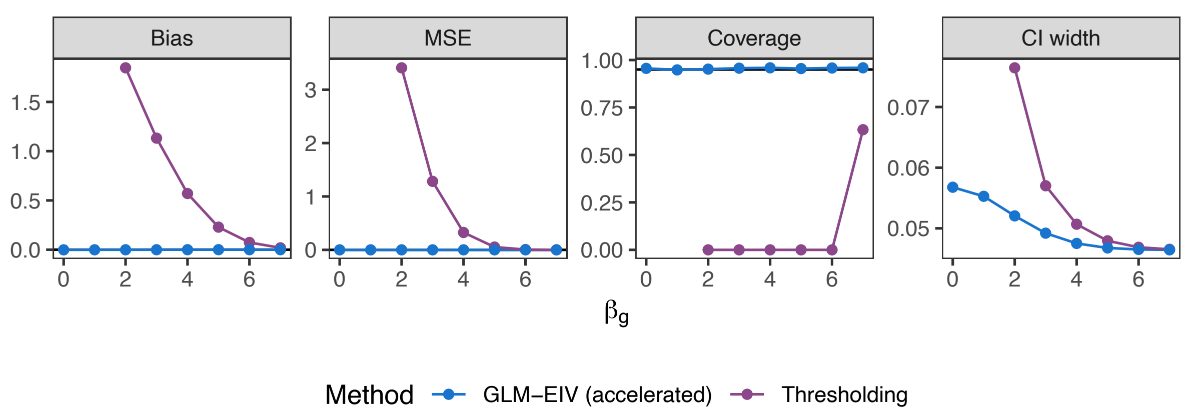

Figure 10: Additional simulation results on Gaussian data. GLM-EIV (accelerated) outperformed the thresholding method on bias, mean squared error, confidence interval coverage rate, and confidence interval width metrics.

F Data analysis details

First, we performed quality control on both datasets. As is standard in single-cell analysis, we removed cells with a high fraction ($> 8\%$) of mitochondrial reads (Choudhary and Satija 2021). We additionally excluded genes that were expressed in fewer than 10% of cells or that had a mean expression level of less than 1. We excluded cells in the Gasperini dataset with gene transcript UMI or gRNA counts below the 5th percentile or above the 95th percentile to reduce the effect of outliers. We did not repeat this latter quality control step on the Xie data because the Xie data were less noisy. The quality-controlled Gasperini and Xie datasets contained $n = 170,645$ (resp. $n = 101,508$) cells, $2,079$ (resp. $1,030$) genes, and $6,598$ (resp. 516) distinct perturbations.

The Gasperini dataset came with $17,028$ candidate *cis* pairs, $97,818$ negative control pairs, and 322 positive control pairs. The *cis* pairs consisted of genes paired to nearby enhancers with unknown regulatory effects. The negative control pairs consisted of non-targeting gRNAs paired to genes. The positive control pairs are described in the main text. The Xie data did not come with either *cis*, negative control, or positive control pairs. Therefore, we constructed a set of 681 candidate *cis* pairs by pairing perturbations to nearby genes, and we constructed a set of $50,000$ *in silico* negative control by pairing perturbations to genes on different chromosomes. See the *Methods* section of Barry et al. (2021) for details on the construction of *cis* and *in silico* negative control pairs on the Xie data.

We modeled the gene expression counts using a negative binomial distributions with unknown size parameter θ; we estimated θ using the glm.nb package. Choudhary and Satija (2021) report that Poisson models accurately capture highly sparse single-cell data.

Although Choudhary and Satija did not investigate the application of Poisson models gRNA data specifically, we modeled the gRNA counts using Poisson distributions, as the gRNA modality exhibited greater sparsity than the gene modality.

We applied GLM-EIV and the thresholding method to analyze the entire set of pairs in both datasets. We did not report results on the candidate *cis* pairs in the text because we do not know the ground truth for these pairs, making them less useful for method assessment. We focused our attention instead on the negative control pairs in both datasets and the positive control pairs in the Gasperini dataset (Figures 2 and 4).

We describe in more detail how we conducted the "excess background contamination" analysis (Figure 4, panels c-f). For each positive control pair, we varied excess background contamination over the grid $[0.0, 0.05, 0.1, \ldots, 0.4]$. For a given level of excess background contamination, we generated $B = 50$ synthetic gRNA datasets, holding fixed the raw gene expressions, covariates, library sizes, and fitted perturbation probabilities. We fitted GLM-EIV and the thresholding method to the data, yielding estimates $[\hat{\beta}_1^m]^{(1)}, \ldots, [\hat{\beta}_1^m]^{(B)}$. Next, we averaged over the $[\hat{\beta}_1^m]^{(i)}$s to obtain the mean estimate for a given pair and level of background contamination, and we calculated the REC using these mean estimates.

References

Barry, Timothy et al. (2021). "SCEPTRE improves calibration and sensitivity in single-cell CRISPR screen analysis". In: *Genome Biology*, pp. 1–19.

Choudhary, Saket and Rahul Satija (2021). "Comparison and evaluation of statistical error models for scRNA-seq". In: *bioRxiv* 8, p. 2021.07.07.451498.

Fitzpatrick, Patrick (2009). *Advanced calculus*. Vol. 5. American Mathematical Soc.

Louis, By Thomas A (1982). “Finding the Observed Information Matrix when Using the EM Algorithm”. In: *Journal of the Royal Statistical Society, Series B* 44.2, pp. 226–233.