Supplement 1

Fraction of oxygenated haemoglobin (FO2Hb)

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Contents

1 Research question

1. Determine whether there is an association between blood colour components (CIE L*a*b*) and Hb saturation.

2. Generate a predictive model of the (CIE L*a*b*) colour component from Hb saturation.

---

2 Load data

```
data <- read_rds('data-cleaned/clean-data.rds')
```

---

3 Quick look at the data

```
dim(data)
```

```
## [1] 163  22
```

```
names(data)
```

```
##  [1] "Animal_ID"        "Trial"            "Dpat"
##  [4] "Time_min"         "l_star"           "a_star"
##  [7] "b_star"           "PaO2"             "cal_SaO2"
## [10] "PaCO2"            "Cl"               "HCO3"
## [13] "Temp"             "pH"               "Cartridge_expired"
## [16] "Coox_SaO2"        "Hct"              "total_Hb"
## [19] "O2Hb"             "COHb"             "MetHb"
## [22] "O2_suppl"
```

```
glimpse(data)
```

```
## Rows: 163
## Columns: 22
## $ Animal_ID        <chr> "I1", "I1", "I1", "I1", "I1", "I1", "I1", "I1", "...
## $ Trial            <dbl> 1, 1, 1, 1, 1, 4, 4, 4, 4, 4, 5, 5, 5, 5, 5, 5, 5...
## $ Dpat             <dbl> 0, 0, 0, 0, 0, 5, 5, 5, 5, 5, 0, 0, 0, 0, 0, 0, 0...
## $ Time_min         <dbl> 5, 10, 15, 20, 30, 5, 10, 15, 20, 30, 5, 10, 15, ...
## $ l_star           <dbl> 11.3, 12.9, 13.5, 14.6, 15.6, 12.6, 13.6, 13.8, 1...
## $ a_star           <dbl> 24.5, 31.5, 31.4, 34.1, 34.8, 30.7, 33.0, 31.4, 3...
## $ b_star           <dbl> 11.5, 17.5, 14.7, 19.9, 20.1, 16.5, 18.1, 18.2, 2...
## $ PaO2             <dbl> 27.9, 40.4, 35.3, 41.7, 40.3, 33.9, 34.9, 36.5, 4...
## $ cal_SaO2         <dbl> 45.6, 71.0, 62.3, 73.4, 71.9, 56.7, 60.1, 61.5, 7...
## $ PaCO2            <dbl> 54.9, 55.6, 58.5, 56.3, 57.6, 63.4, 62.4, 63.8, 6...
## $ Cl               <dbl> 113, 113, 111, 111, 109, 107, 106, 106, 105, 105,...
## $ HCO3             <dbl> 27.9, 29.9, 31.3, 31.3, 33.0, 31.0, 32.0, 31.0, 3...
## $ Temp             <dbl> 39.2, 39.7, 39.7, 39.7, 39.6, 39.2, 39.6, 39.6, 3...
## $ pH               <dbl> 7.31, 7.34, 7.34, 7.35, 7.37, 7.30, 7.32, 7.29, 7...
## $ Cartridge_expired <chr> "No", "No", "No", "No", "No", "No", "No", "No", "...
## $ Coox_SaO2        <dbl> 53.0, 67.8, 63.3, 74.1, 73.1, 53.8, 64.2, 56.2, 7...
## $ Hct              <dbl> 23, 24, 21, 20, 18, 27, 23, 26, 20, 19, 21, 20, 1...
## $ total_Hb         <dbl> 8.7, 7.8, 7.6, 7.2, 6.7, 9.6, 8.9, 9.8, 7.7, 7.3,...
## $ O2Hb             <dbl> 51.0, 65.4, 60.0, 71.0, 69.4, 51.9, 61.7, 54.2, 6...
## $ COHb             <dbl> 0.0, 1.2, 2.4, 1.0, 1.7, 0.0, 0.8, 0.0, 1.5, 1.4,...
## $ MetHb            <dbl> 3.7, 2.3, 2.8, 3.2, 3.3, 3.4, 3.1, 3.4, 2.4, 2.9,...
## $ O2_suppl         <chr> "No", "No", "No", "No", "No", "No", "No", "No", "...
```

```
data[, !(names(data) %in% c('Trial'))] %>%
    select_if(is.numeric) %>%
    my_skim()
```

Table 1: Data summary

Name	Piped data
Number of rows	163
Number of columns	18
Column type frequency:	
numeric	18
Group variables	None

Variable type: numeric

skim_variable	n_missing	complete_rate	mean	sd	p0	p25	p50	p75	p100
Dpat	0	1.00	10.67	22.33	0.00	0.00	0.00	5.00	70.0
Time_min	0	1.00	22.37	14.21	5.00	10.00	20.00	30.00	50.0
l_star	0	1.00	17.53	2.78	9.20	16.00	18.10	19.40	22.0
a_star	0	1.00	38.97	5.58	19.80	35.90	40.00	42.55	47.8
b_star	0	1.00	24.95	5.75	7.50	21.35	25.50	28.80	36.0
PaO2	7	0.96	85.66	73.43	7.20	44.00	56.55	70.32	311.3
cal_SaO2	15	0.91	82.25	16.17	15.50	74.25	86.80	93.17	99.9
PaCO2	6	0.96	52.57	8.50	33.50	46.20	51.90	57.60	79.6
Cl	6	0.96	110.18	3.14	105.00	108.00	110.00	112.00	121.0
HCO3	14	0.91	29.64	3.27	22.30	27.40	29.30	32.00	37.3
Temp	7	0.96	39.01	0.83	37.00	38.70	39.10	39.50	41.2
pH	14	0.91	7.36	0.07	7.11	7.32	7.37	7.41	7.5
Coox_SaO2	0	1.00	82.49	14.76	16.30	76.20	87.50	92.00	99.3
Hct	8	0.95	23.87	4.52	15.00	20.00	24.00	26.00	38.0
total_Hb	0	1.00	8.86	1.49	5.70	7.70	8.80	9.60	13.4
O2Hb	0	1.00	79.39	14.08	15.70	73.75	83.90	88.50	98.1
COHb	0	1.00	1.79	1.15	0.00	0.90	1.70	2.60	4.7
MetHb	0	1.00	1.98	0.68	0.30	1.45	2.00	2.40	3.8

4 Prepare data

```
# Convert Animal ID to a factor
data %<>%
    mutate(Animal_ID = factor(Animal_ID,
                       levels = c('I1', 'I2', 'I3', 'I4', 'I5',
                             'I6', 'I8', 'I9', 'I10', 'I11')))
```

5 L*

5.1 Exploratory plots

5.1.1 Linear model

```r
# Composite plot
ggplot(data = data) +
    aes(y = l_star,
        x = O2Hb,
        fill = Animal_ID) +
    geom_point(shape = 21,
               size = 3) +
    geom_smooth(se = FALSE,
                size = 0.8,
                method = 'lm',
                fill = 'blue') +
    scale_fill_viridis_d(name = 'Animal') +
    labs(title = 'FO2Hb vs L*',
         subtitle = 'All animals together\nRegression line is simple linear model',
         x = 'FO2Hb (%)',
         y = 'L*')
```


FO2Hb vs L*

All animals together
Regression line is simple linear model

```r
# Small multiples
ggplot(data = data) +
    aes(y = l_star,
        x = O2Hb) +
    geom_point(shape = 21,
               size = 3,
               fill = '#FFFFFF') +
    geom_smooth(se = FALSE,
                size = 0.8,
                method = 'lm') +
    labs(title = 'FO2Hb vs L*',
         subtitle = 'Facetted by animal\nRegression line: linear',
         y = 'L*',
         x = 'FO2Hb (%)') +
    facet_wrap(~ Animal_ID,
               ncol = 3)
```

FO2Hb vs L*

Facetted by animal
Regression line: linear

5.1.2 Quadratic model
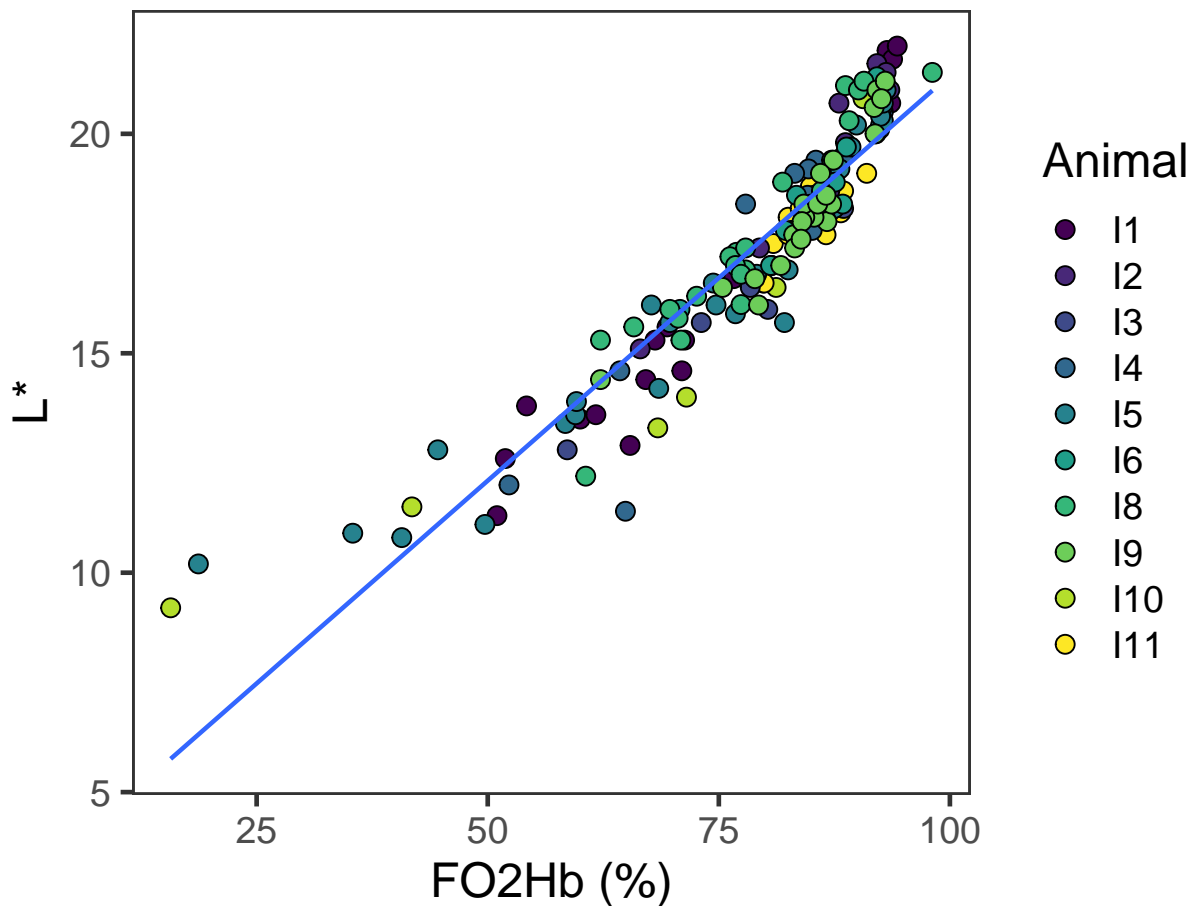
```r
# Composite plot
ggplot(data = data) +
    aes(y = l_star,
        x = O2Hb,
        fill = Animal_ID) +
    geom_point(shape = 21,
               size = 3) +
```

```
        geom_smooth(se = FALSE,
                    size = 0.8,
                    method = 'lm',
                    fill = 'blue',
                    formula = y ~ poly(x, 2)) +
        scale_fill_viridis_d(name = 'Animal') +
        labs(title = 'FO2Hb vs L*',
             subtitle = 'All animals together\nRegression line is a quadratic polynomial model',
             x = 'FO2Hb (%)',
             y = 'L*')
```

FO2Hb vs L*

All animals together
Regression line is a quadratic polynomial model
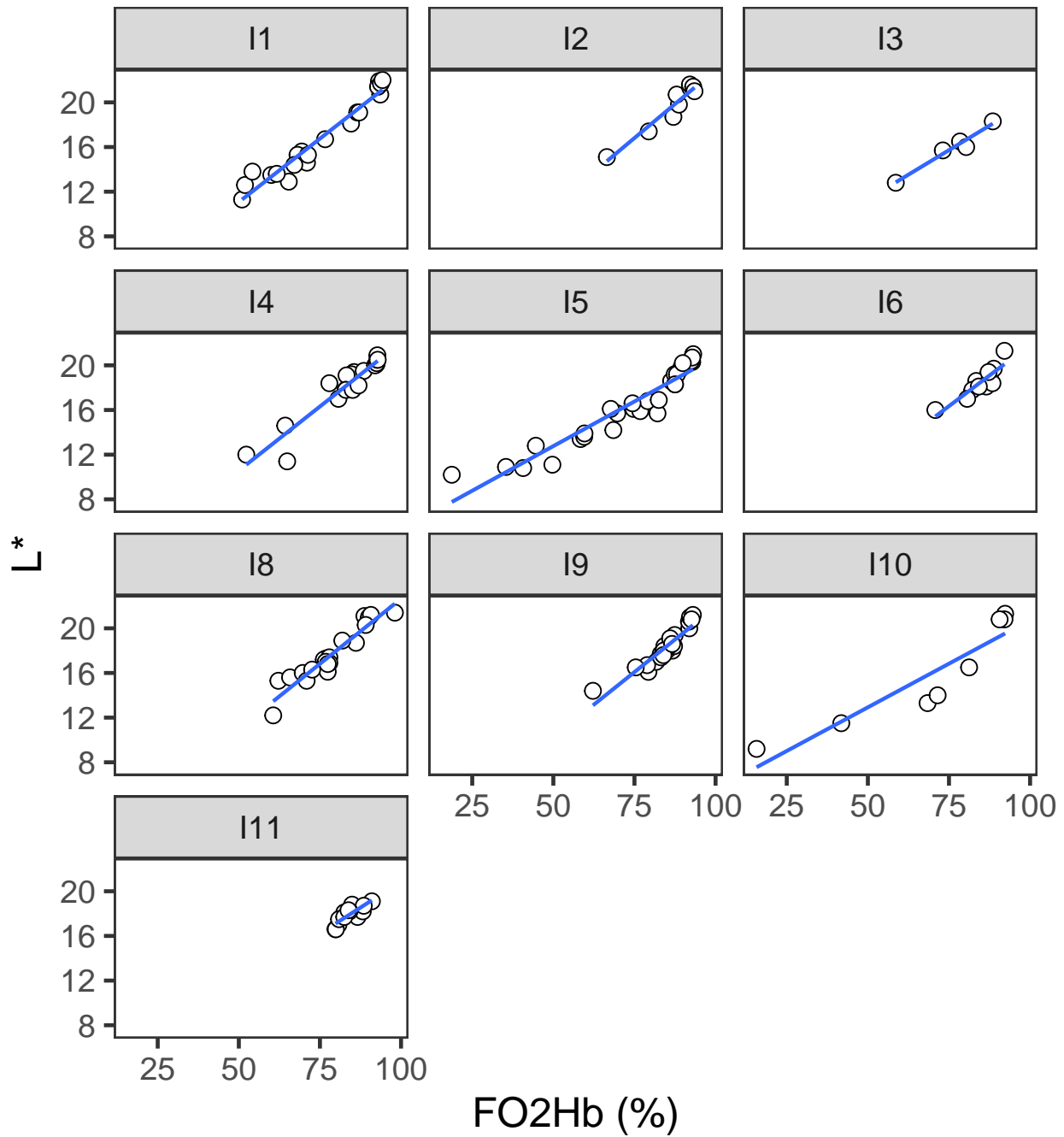


```
# Small multiples
ggplot(data = data) +
    aes(y = l_star,
        x = O2Hb) +
    geom_point(shape = 21,
               size = 3,
               fill = '#FFFFFF') +
    geom_smooth(se = FALSE,
                size = 0.8,
                colour = 'blue',
                method = 'lm',
                formula = y ~ poly(x, 2, raw = TRUE)) +
    labs(title = 'FO2Hb vs L*',
```

```
          subtitle = 'Facetted by animal\nRegression line: quadratic polynomial',
          y = 'L*',
          x = 'FO2Hb (%)') +
    facet_wrap(~ Animal_ID,
               ncol = 3)
```

FO2Hb vs L*

Facetted by animal
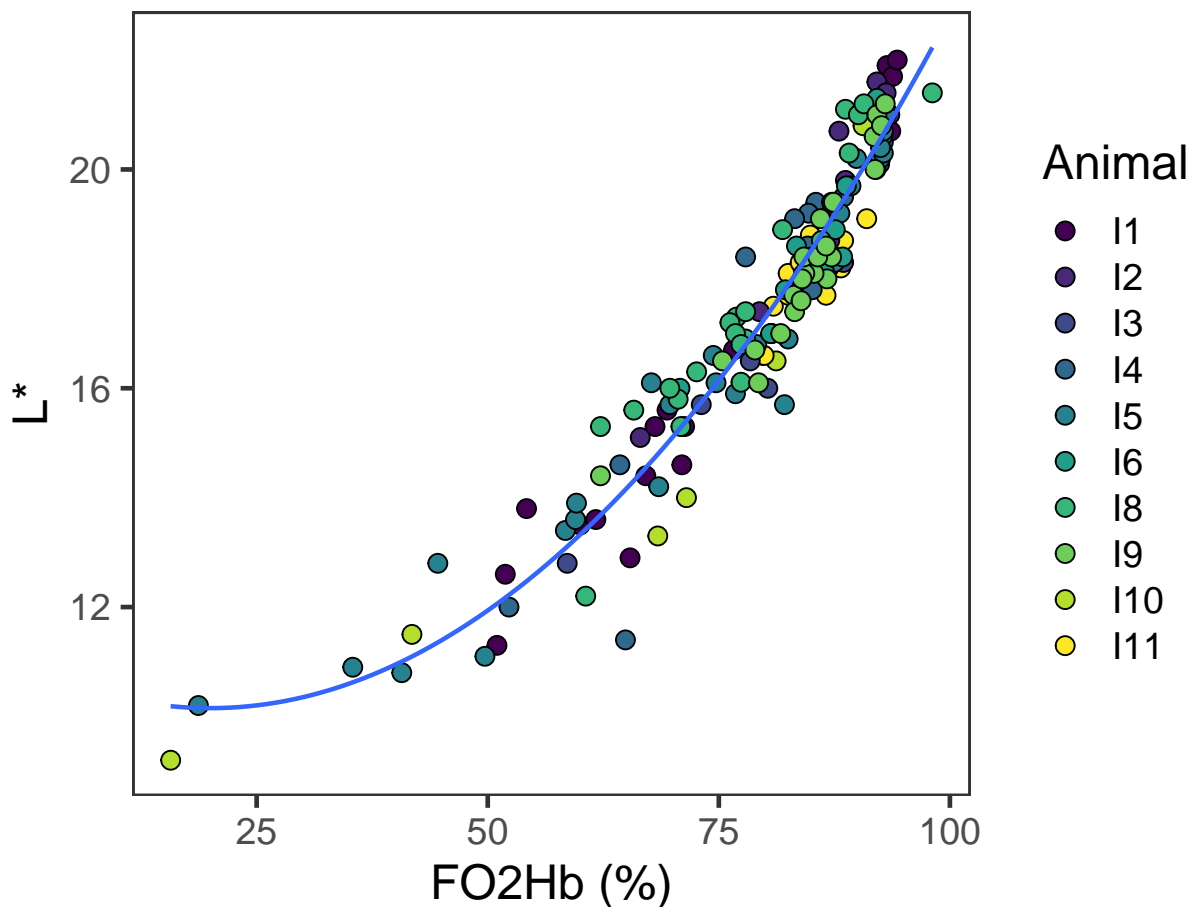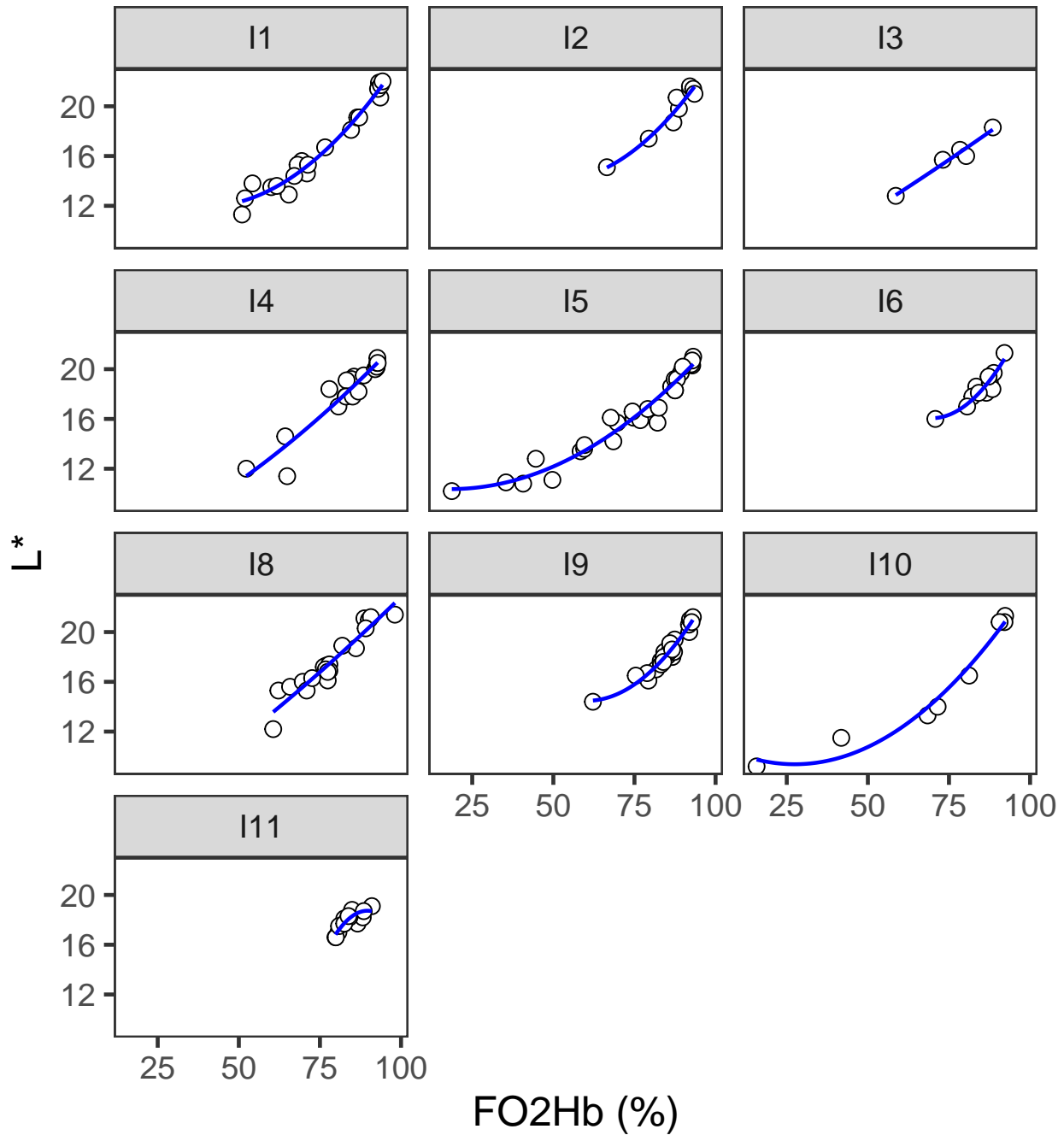Regression line: quadratic polynomial

FO2Hb (%)

5.2 Model selection

Model selection based on the outcome of likelihood ratio test of goodness of fit.

```
# Null model
lstar_null <- lmer(l_star ~ 1 + (1 | Animal_ID),
                   data = data,
                   REML = FALSE)

## First-order polynomial (linear) model
lstar_linear <- lmer(l_star ~ O2Hb + (1 | Animal_ID),
                     data = data,
                     REML = FALSE)

## Second-order polynomial (quadratic) model
lstar_poly <- lmer(l_star ~ poly(O2Hb, 2) + (1 | Animal_ID),
                   data = data,
                   REML = FALSE)

# Compare model fits
anova(lstar_null, lstar_linear)
```

```
## Data: data
## Models:
## lstar_null: l_star ~ 1 + (1 | Animal_ID)
## lstar_linear: l_star ~ O2Hb + (1 | Animal_ID)
##              Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## lstar_null    3 798.66 807.94 -396.33   792.66
## lstar_linear  4 465.72 478.09 -228.86   457.72 334.94      1  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(lstar_null, lstar_poly)
```

```
## Data: data
## Models:
## lstar_null: l_star ~ 1 + (1 | Animal_ID)
## lstar_poly: l_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
##             Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## lstar_null   3 798.66 807.94 -396.33   792.66
## lstar_poly   5 362.57 378.04 -176.29   352.57 440.08      2  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(lstar_linear, lstar_poly)
```

```
## Data: data
## Models:
## lstar_linear: l_star ~ O2Hb + (1 | Animal_ID)
## lstar_poly: l_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
##               Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## lstar_linear   4 465.72 478.09 -228.86   457.72
## lstar_poly     5 362.57 378.04 -176.29   352.57 105.15      1  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Best fit model is the second-order polynomial (quadratic) model.

5.3   Explore the best fit model

```
# Summary output
summary(lstar_poly)
```

```
## Linear mixed model fit by maximum likelihood . t-tests use Satterthwaite's
##   method [lmerModLmerTest]
## Formula: l_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
##    Data: data
##
##      AIC      BIC   logLik deviance df.resid
##    362.6    378.0   -176.3    352.6      158
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.8498 -0.5328  0.0333  0.6422  2.4622
##
## Random effects:
##  Groups    Name        Variance Std.Dev.
##  Animal_ID (Intercept) 0.07597  0.2756
##  Residual              0.47238  0.6873
## Number of obs: 163, groups:  Animal_ID, 10
##
## Fixed effects:
##               Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)    17.5055     0.1052   8.4061  166.33 4.39e-16 ***
## poly(O2Hb, 2)1 33.2314     0.7236 161.9920   45.92  < 2e-16 ***
## poly(O2Hb, 2)2  8.7227     0.7095 160.3221   12.29  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) p(O2H,2)1
## ply(O2H,2)1 -0.007
## ply(O2H,2)2 -0.008 -0.014
```

```r
# Check model assumptions
plot_model(model = lstar_poly,
           type = 'diag')[2:4]
```
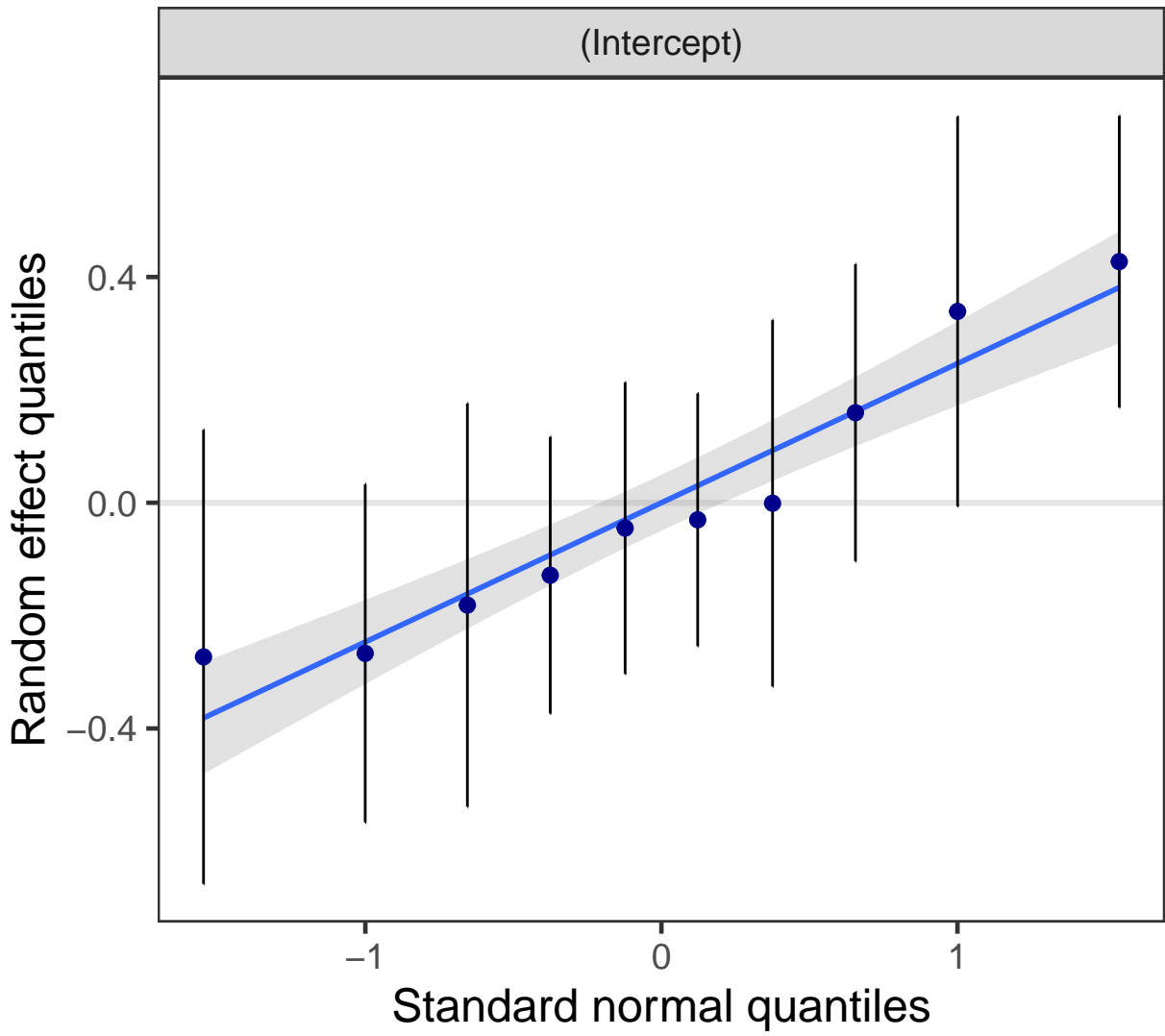
```
## [[1]]
## [[1]]$Animal_ID
```

(Intercept)

Random effect quantiles

Standard normal quantiles

```
##
##
## [[2]]
```

Non−normality of residuals

Distribution should look like normal curve

```
## 
## [[3]]
```
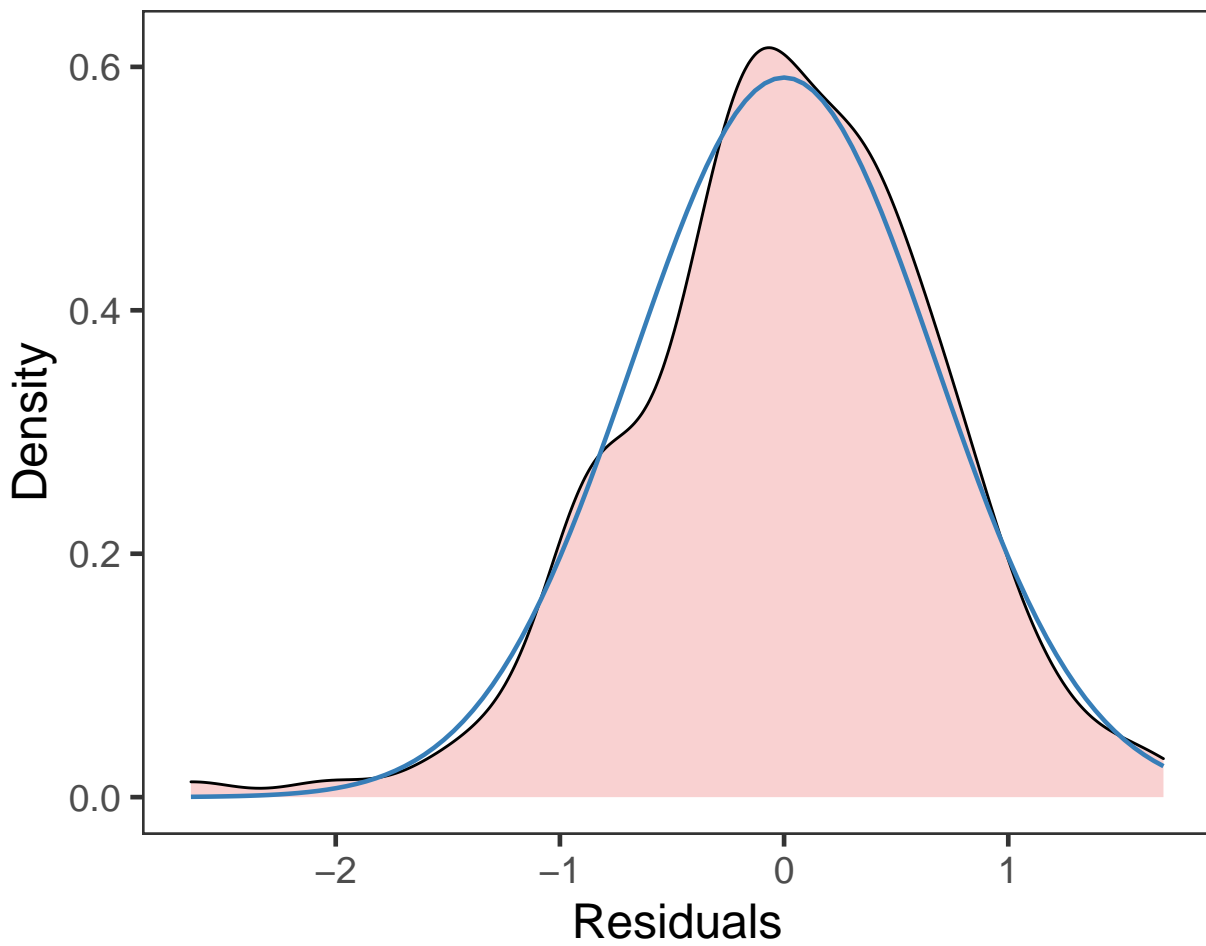
Homoscedasticity (constant variance of residuals)

Amount and distance of points scattered above/below line is equal
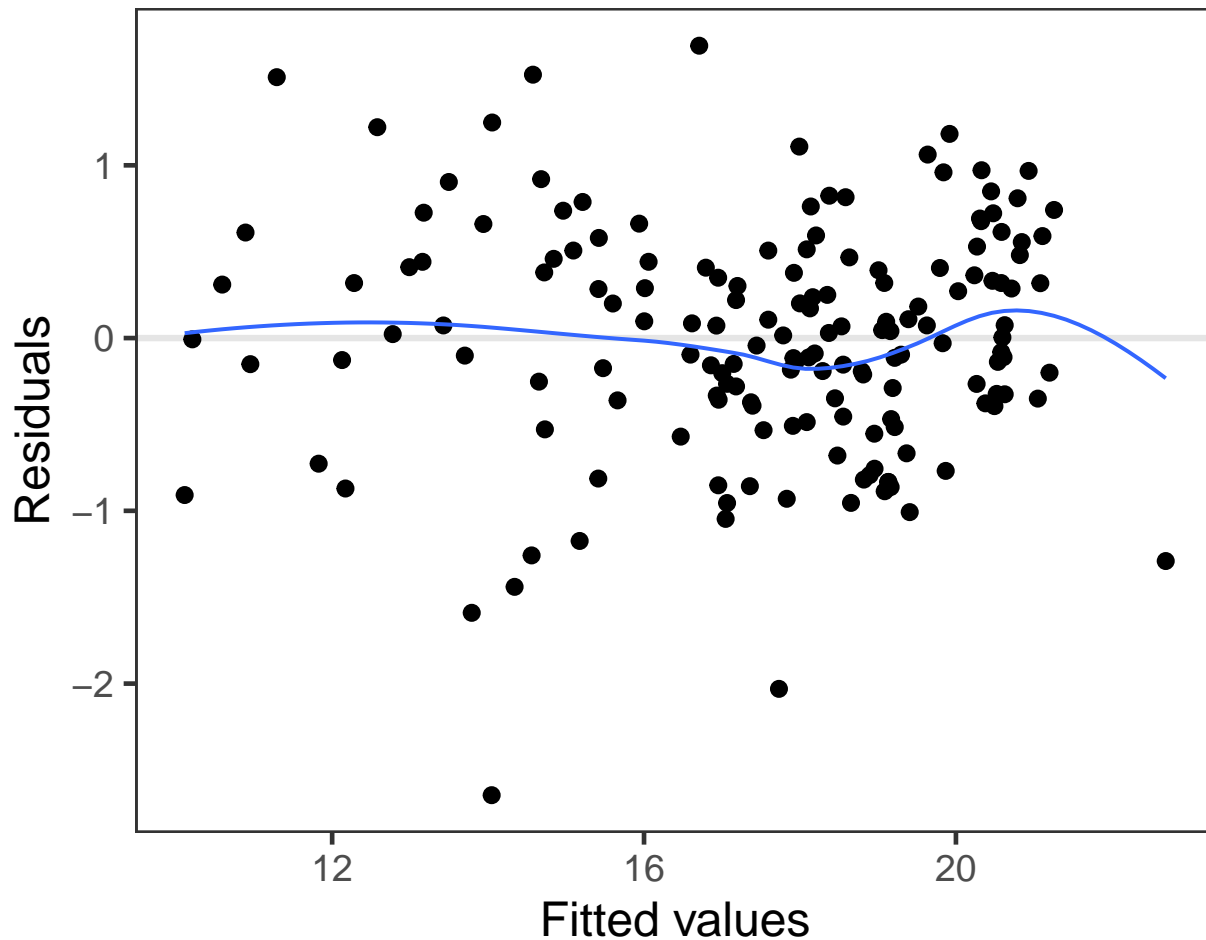

```
# R-squared (based on likelihood ratio test)
r.squaredLR(lstar_poly, null = lstar_null)
```

```
## [1] 0.9327871
## attr(,"adj.r.squared")
## [1] 0.9400518
```

```
# Root mean square error
RMSE.merMod(lstar_poly)
```

```
## [1] 0.6726812
```

5.4 Generate prediction intervals

```
# Prediction interval
lstar_lqmm <- lqmm(fixed = l_star ~ poly(O2Hb, 2),
             random = ~ 1,
             group = ~ Animal_ID,
             tau = c(0.025, 0.976),
             data = data)

lstar_interval <- as.data.frame(predict(lstar_lqmm, level = 0))

names(lstar_interval) <- paste0('Q', c(2.5, 97.5))
```

```
lstar_interval <- cbind(data, lstar_interval)

# Numeric output
lstar_interval %>%
    dplyr::select(O2Hb, l_star, Q2.5, Q97.5) %>%
    arrange(O2Hb) %>%
    unique(.) %>%
    kable(caption = 'L*: 95% prediction interval',
          col.names = c('FO2Hb (%)', 'L*', 'Q2.5', 'Q97.5'))
```

Table 3: L*: 95% prediction interval

|	FO2Hb (%)	L*	Q2.5	Q97.5
1	15.7	9.20	9.104688	11.60561
2	18.7	10.20	9.025719	11.57788
3	35.4	10.90	9.296772	12.06589
4	40.7	10.80	9.634662	12.44844
5	41.8	11.50	9.719997	12.54159
6	44.6	12.80	9.960802	12.80000
7	49.7	11.10	10.486440	13.34936
8	51.0	11.30	10.638399	13.50563
9	51.9	12.60	10.747877	13.61769
10	52.3	12.00	10.797658	13.66851
11	54.2	13.80	11.043555	13.91843
12	58.4	13.40	11.642459	14.52092
13	58.6	12.80	11.672879	14.55132
14	59.5	13.60	11.811908	14.69009
15	59.6	13.90	11.827571	14.70571
16	60.0	13.50	11.890658	14.76855
17	60.6	12.20	11.986584	14.86400
18	61.7	13.60	12.166488	15.04262
19	62.2	15.30	12.249991	15.12538
20	62.2	14.40	12.249991	15.12538
21	64.3	14.60	12.612496	15.48362
22	64.9	11.40	12.719568	15.58914
23	65.4	12.90	12.809983	15.67814
24	65.8	15.60	12.883092	15.75005
25	66.5	15.10	13.012697	15.87739
26	67.1	14.40	13.125472	15.98806
27	67.7	16.10	13.239802	16.10014
28	68.1	15.30	13.316886	16.17564
29	68.4	13.30	13.375153	16.23267
30	68.5	14.20	13.394661	16.25176
31	69.4	15.60	13.572183	16.42533
32	69.7	15.70	13.632135	16.48388
33	69.7	16.00	13.632135	16.48388
34	70.6	15.80	13.814322	16.66167
35	70.8	16.00	13.855284	16.70160
36	70.9	15.30	13.875829	16.72163
37	71.0	14.60	13.896418	16.74170
38	71.3	15.30	13.958443	16.80213
39	71.5	14.00	14.000009	16.84261
40	72.6	16.30	14.231712	17.06806
41	73.1	15.70	14.338760	17.17210
42	74.4	16.60	14.622138	17.44717
43	74.7	16.10	14.688570	17.51159
44	75.4	16.50	14.845089	17.66326
45	76.2	17.20	15.026561	17.83894

	FO2Hb (%)	L*	Q2.5	Q97.5
46	76.6	16.70	15.118334	17.92772
47	76.8	15.90	15.164480	17.97234
48	76.8	17.00	15.164480	17.97234
49	76.9	17.30	15.187617	17.99471
50	77.4	16.11	15.303953	18.10714
51	77.4	16.80	15.303953	18.10714
52	77.9	18.40	15.421369	18.22056
53	77.9	17.40	15.421369	18.22056
54	77.9	16.90	15.421369	18.22056
55	78.4	16.50	15.539865	18.33495
56	78.9	16.70	15.659441	18.45031
57	79.1	16.80	15.707574	18.49673
58	79.3	16.10	15.755880	18.54331
59	79.4	17.40	15.780097	18.56665
60	79.8	16.60	15.877400	18.66043
61	79.9	16.60	15.901834	18.68397
62	80.3	16.00	16.000000	18.77853
63	80.6	17.00	16.074079	18.84985
64	80.7	17.00	16.098858	18.87371
66	80.9	17.50	16.148546	18.92153
67	81.2	16.50	16.223402	18.99356
68	81.7	17.00	16.349027	19.11439
69	81.9	18.90	16.399579	19.16300
70	82.1	15.70	16.450304	19.21176
71	82.2	17.80	16.475731	19.23620
72	82.5	18.10	16.552272	19.30976
73	82.5	17.70	16.552272	19.30976
74	82.5	16.90	16.552272	19.30976
75	82.9	17.80	16.654932	19.40838
76	83.0	17.80	16.680705	19.43313
77	83.1	17.70	16.706521	19.45792
78	83.2	19.10	16.732380	19.48275
79	83.2	17.40	16.732380	19.48275
80	83.4	18.60	16.784228	19.53253
81	83.8	18.30	16.888443	19.63255
82	83.9	17.60	16.914605	19.65765
83	84.0	18.00	16.940810	19.68280
84	84.1	18.20	16.967058	19.70798
85	84.2	18.10	16.993349	19.73320
86	84.2	18.40	16.993349	19.73320
87	84.3	18.10	17.019683	19.75846
88	84.6	18.10	17.098946	19.83447
89	84.6	18.30	17.098946	19.83447
90	84.6	18.60	17.098946	19.83447
91	84.7	19.20	17.125454	19.85989
92	84.9	18.80	17.178598	19.91084
93	85.0	18.40	17.205235	19.93637
94	85.1	17.80	17.231915	19.96194
95	85.3	18.60	17.285405	20.01321
96	85.3	18.10	17.285405	20.01321
97	85.5	19.40	17.339067	20.06462
98	85.7	18.40	17.392903	20.11620
99	86.0	19.10	17.473980	20.19385
100	86.1	18.70	17.501092	20.21981
101	86.3	18.60	17.555446	20.27186
102	86.5	19.10	17.609973	20.32406
103	86.5	18.10	17.609973	20.32406

	FO2Hb (%)	L*	Q2.5	Q97.5
104	86.6	17.70	17.637301	20.35021
105	86.6	18.60	17.637301	20.35021
106	86.7	18.00	17.664673	20.37641
107	86.9	18.20	17.719545	20.42892
108	87.0	18.70	17.747046	20.45524
109	87.1	19.10	17.774590	20.48159
110	87.2	19.40	17.802177	20.50798
111	87.2	18.40	17.802177	20.50798
112	87.4	19.20	17.857482	20.56088
113	87.4	19.40	17.857482	20.56088
114	87.5	18.30	17.885199	20.58739
116	87.6	19.20	17.912959	20.61394
117	87.6	18.90	17.912959	20.61394
118	88.0	20.70	18.024432	20.72053
119	88.1	19.20	18.052408	20.74727
120	88.2	18.20	18.080427	20.77405
121	88.4	18.40	18.136596	20.82774
122	88.5	18.70	18.164745	20.85464
123	88.5	18.30	18.164745	20.85464
124	88.5	19.50	18.164745	20.85464
125	88.7	19.80	18.221172	20.90855
126	88.7	21.10	18.221172	20.90855
127	88.8	19.70	18.249451	20.93557
128	89.1	20.30	18.334546	21.01686
129	89.3	19.70	18.391492	21.07124
130	89.9	20.20	18.563367	21.23534
131	90.1	21.00	18.621004	21.29035
132	90.6	20.80	18.765853	21.42856
133	90.7	21.20	18.794953	21.45632
134	91.0	19.10	18.882510	21.53983
135	91.8	20.60	19.117898	21.76425
136	91.9	20.00	19.147516	21.79247
137	92.0	20.00	19.177177	21.82074
138	92.1	20.80	19.206881	21.84905
139	92.1	21.60	19.206881	21.84905
140	92.1	21.30	19.206881	21.84905
141	92.1	21.00	19.206881	21.84905
142	92.2	21.30	19.236629	21.87739
143	92.3	21.30	19.266419	21.90578
144	92.4	20.10	19.296253	21.93420
145	92.5	20.20	19.326130	21.96266
146	92.5	20.40	19.326130	21.96266
147	92.6	20.80	19.356050	21.99116
148	92.7	20.50	19.386014	22.01970
149	92.7	20.90	19.386014	22.01970
150	92.7	20.60	19.386014	22.01970
151	92.8	20.50	19.416021	22.04828
152	92.8	20.30	19.416021	22.04828
153	92.8	20.70	19.416021	22.04828
154	92.9	21.40	19.446071	22.07690
155	93.0	21.20	19.476164	22.10556
156	93.1	21.40	19.506300	22.13425
157	93.1	21.00	19.506300	22.13425
158	93.2	21.90	19.536479	22.16299
159	93.5	21.00	19.627277	22.24943
160	93.6	20.70	19.657629	22.27832
161	93.8	21.70	19.718463	22.33622

	FO2Hb (%)	L*	Q2.5	Q97.5
162	94.3	22.00	19.871305	22.48166
163	98.1	21.40	21.068196	23.61886

```r
# Plot output
ggplot(data = lstar_interval) +
    aes(x = O2Hb) +
    geom_point(data = data,
            aes(y = l_star,
                x = O2Hb,
                fill = Animal_ID),
            shape = 21,
            size = 3) +
    geom_smooth(data = lstar_interval,
            aes(y = `Q2.5`),
            se = FALSE) +
    geom_smooth(data = lstar_interval,
            aes(y = `Q97.5`),
            se = FALSE) +
    scale_fill_viridis_d(name = 'Animal') +
    labs(title = 'FO2Hb vs L*',
        subtitle = '95% prediction interval',
        y = 'L*',
        x = 'FO2Hb (%)')
```

FO2Hb vs L*

95% prediction interval
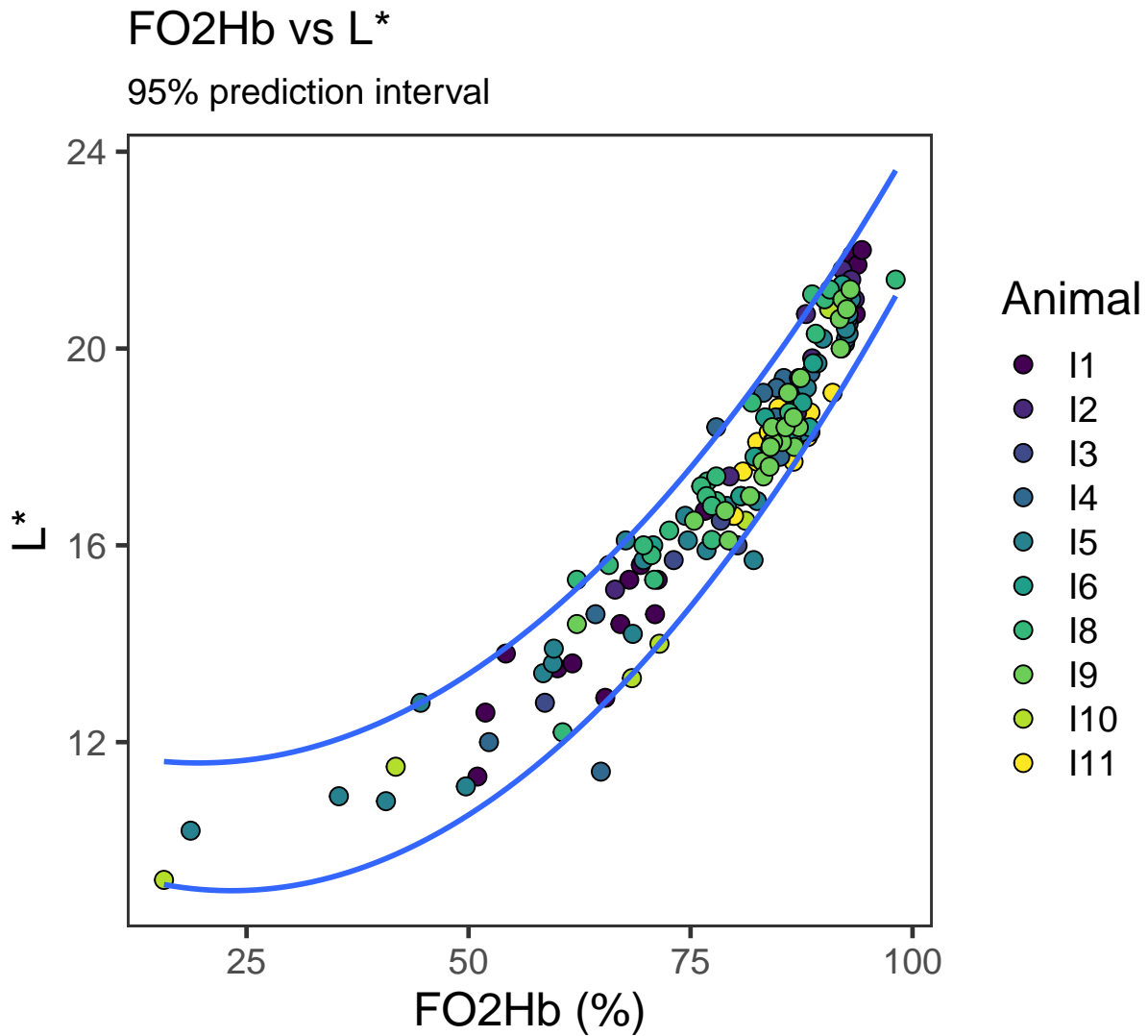
5.5 Generate predicted values

```
# New data
lstar_grid <- expand.grid(O2Hb = c(15, 25, 35, 45, 55, 65, 75, 85, 95),
                          Animal_ID = 'I12')

# Generate predicted vs independent variable O2Hb
lstar_predicted <- predict(lstar_poly,
                           lstar_grid,
                           allow.new.levels = TRUE)

# Tabulate
data.frame(O2Hb = lstar_grid$O2Hb,
           lstar = lstar_predicted) %>%
    kable(caption = 'L* predicted values',
          col.names = c('FO2Hb (%)', 'L*'))
```

Table 4: L* predicted values

FO2Hb (%)	L*
15	10.30745
25	10.24781

FO2Hb (%)	L*
35	10.59812
45	11.35838
55	12.52859
65	14.10876
75	16.09887
85	18.49894
95	21.30895

6 a*

6.1 Exploratory plots

6.1.1 Linear model

```r
# Composite plot
ggplot(data = data) +
    aes(y = a_star,
        x = O2Hb,
        fill = Animal_ID) +
    geom_point(shape = 21,
               size = 3) +
    geom_smooth(se = FALSE,
                size = 0.8,
                method = 'lm',
                fill = 'blue') +
    scale_fill_viridis_d(name = 'Animal') +
    labs(title = 'FO2Hb vs a*',
         subtitle = 'All animals together\nRegression line is simple linear model',
         x = 'FO2Hb (%)',
         y = 'a*')
```

FO2Hb vs a*

All animals together
Regression line is simple linear model


```r
# Small multiples
ggplot(data = data) +
    aes(y = a_star,
        x = O2Hb) +
    geom_point(shape = 21,
               size = 3,
               fill = '#FFFFFF') +
    geom_smooth(se = FALSE,
                size = 0.8,
                method = 'lm') +
    labs(title = 'FO2Hb vs a*',
         subtitle = 'Facetted by animal\nRegression line: linear',
         y = 'a*',
         x = 'FO2Hb (%)') +
    facet_wrap(~ Animal_ID,
               ncol = 3)
```

FO2Hb vs a*

Facetted by animal
Regression line: linear

FO2Hb (%)
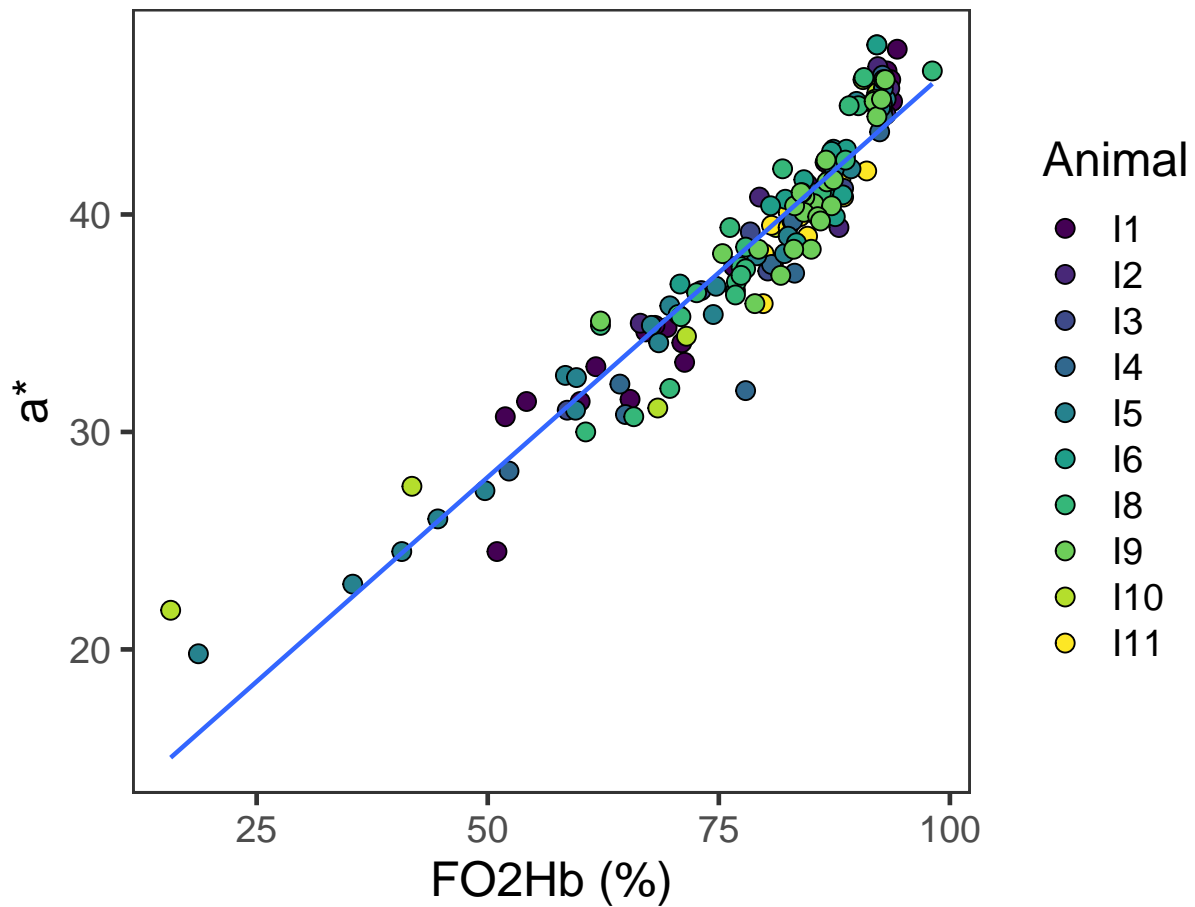
6.1.2 Quadratic model

```
# Composite plot
ggplot(data = data) +
    aes(y = a_star,
        x = O2Hb,
        fill = Animal_ID) +
    geom_point(shape = 21,
            size = 3) +
```

```
    geom_smooth(se = FALSE,
                size = 0.8,
                method = 'lm',
                fill = 'blue',
                formula = y ~ poly(x, 2)) +
    scale_fill_viridis_d(name = 'Animal') +
    labs(title = 'FO2Hb vs a*',
         subtitle = 'All animals together\nRegression line is a quadratic polynomial model',
       x = 'FO2Hb (%)',
       y = 'a*')
```

FO2Hb vs a*

All animals together
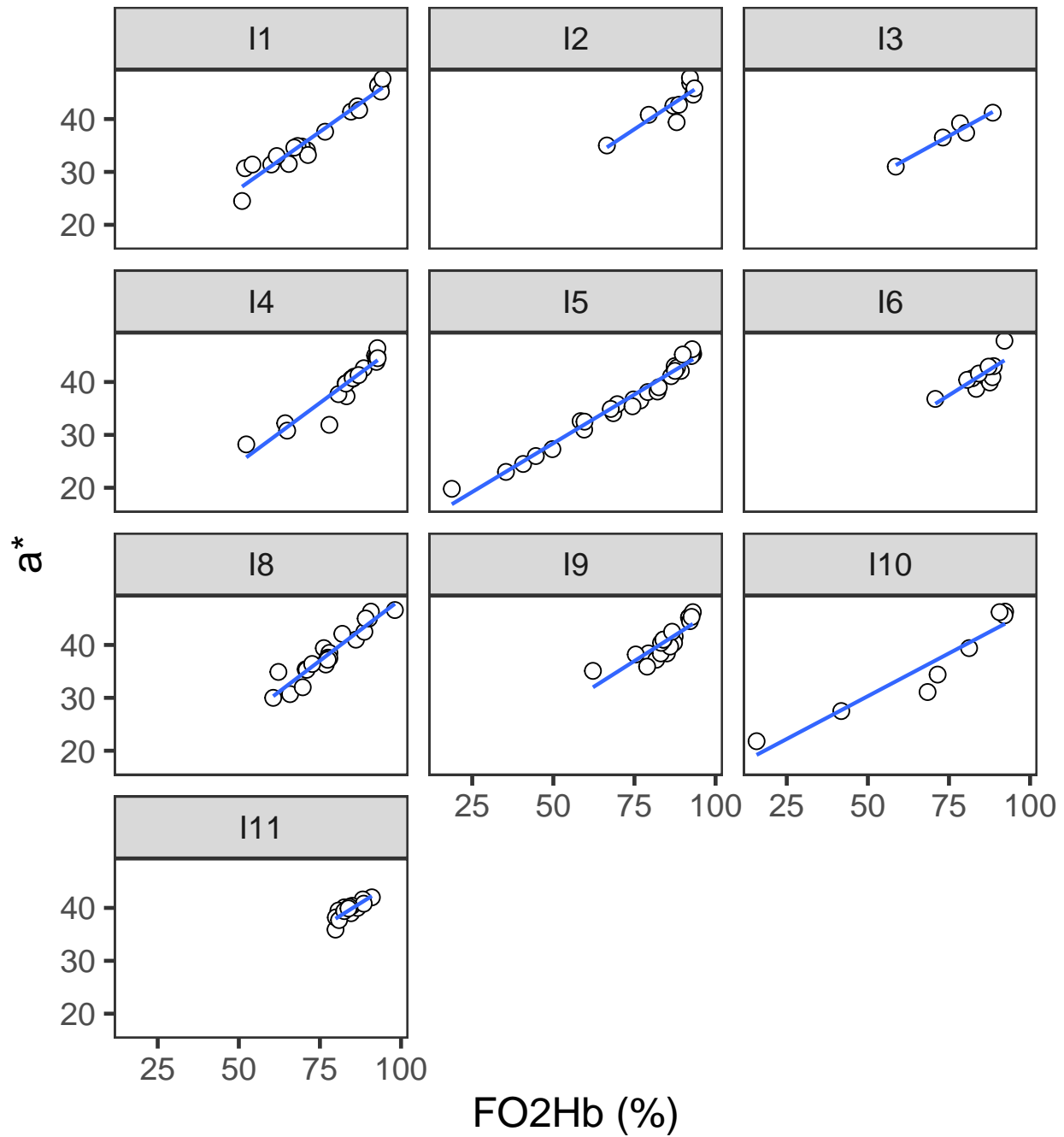Regression line is a quadratic polynomial model


```
# Small multiples
ggplot(data = data) +
    aes(y = a_star,
        x = O2Hb) +
    geom_point(shape = 21,
               size = 3,
               fill = '#FFFFFF') +
    geom_smooth(se = FALSE,
                size = 0.8,
                colour = 'blue',
                method = 'lm',
                formula = y ~ poly(x, 2, raw = TRUE)) +
    labs(title = 'FO2Hb vs a*',
```

```
        subtitle = 'Facetted by animal\nRegression line: quadratic polynomial',
        y = 'a*',
        x = 'FO2Hb (%)') +
    facet_wrap(~ Animal_ID,
               ncol = 3)
```

FO2Hb vs a*

Facetted by animal
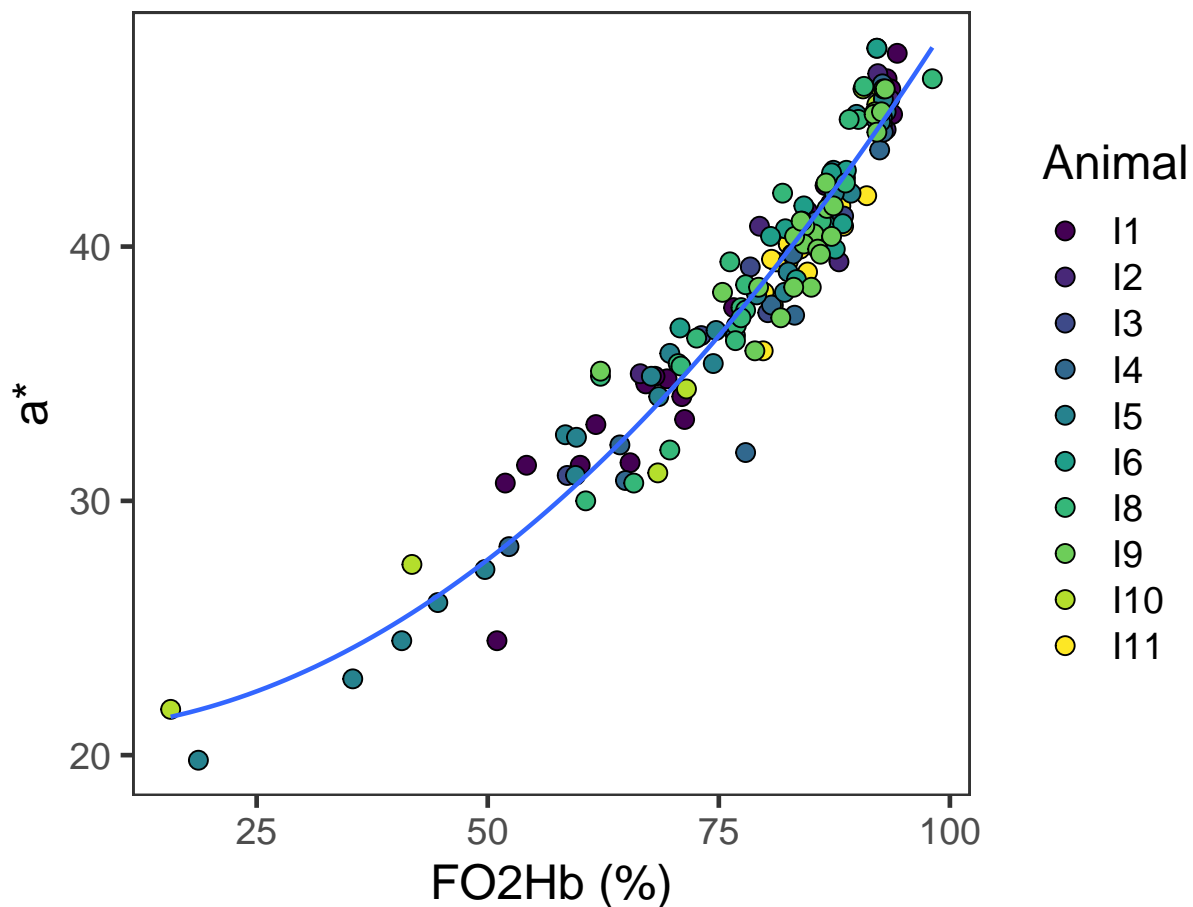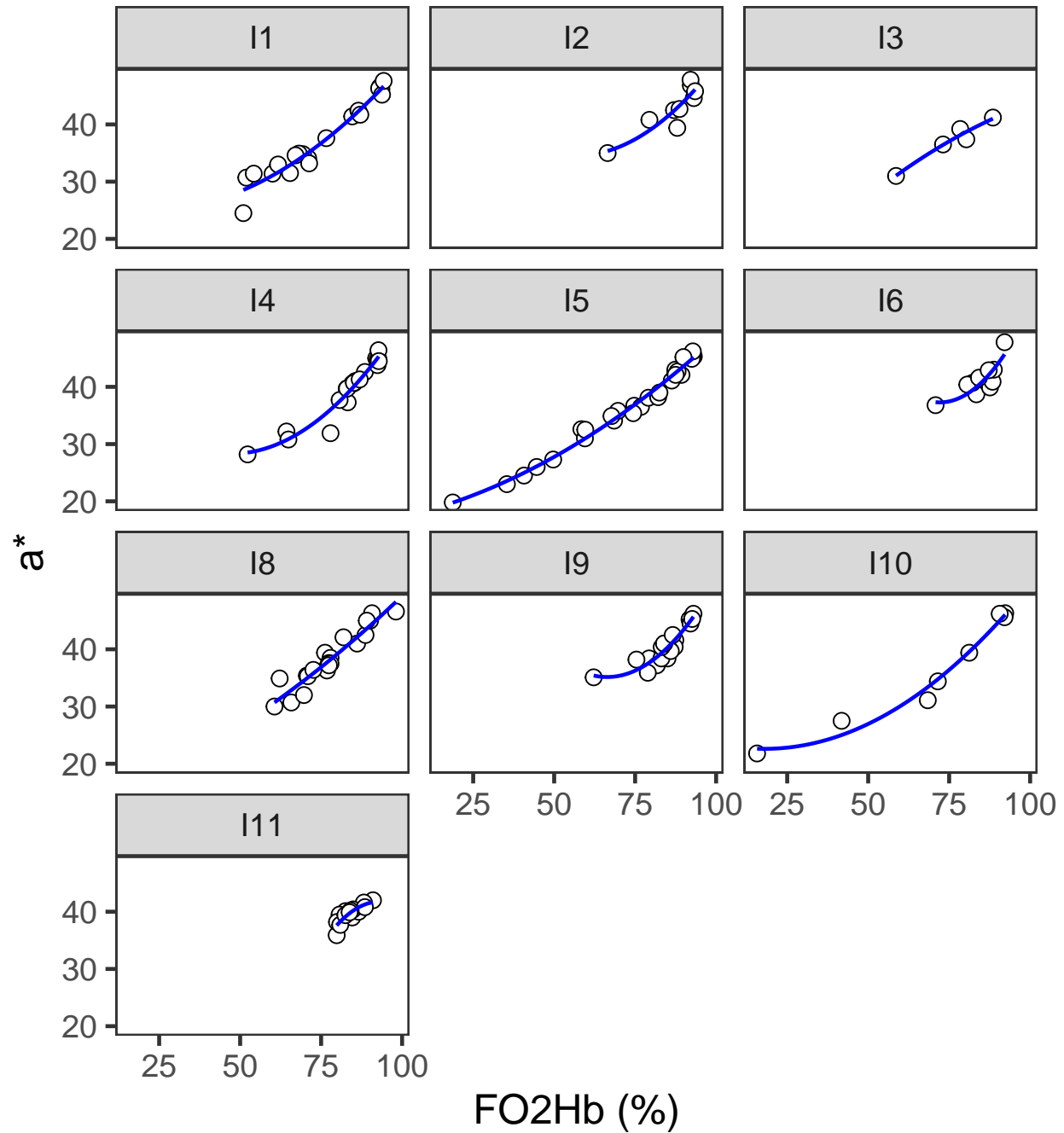Regression line: quadratic polynomial

FO2Hb (%)

6.2 Model selection

Model selection based on the outcome of likelihood ratio test of goodness of fit.

```r
# Null model
astar_null <- lmer(a_star ~ 1 + (1 | Animal_ID),
                   data = data,
                   REML = FALSE)

## First-order polynomial (linear) model
astar_linear <- lmer(a_star ~ O2Hb + (1 | Animal_ID),
                   data = data,
                   REML = FALSE)

## Second-order polynomial (quadratic) model
astar_poly <- lmer(a_star ~ poly(O2Hb, 2) + (1 | Animal_ID),
                   data = data,
                   REML = FALSE)

# Compare model fits
anova(astar_null, astar_linear)
```

```
## Data: data
## Models:
## astar_null: a_star ~ 1 + (1 | Animal_ID)
## astar_linear: a_star ~ O2Hb + (1 | Animal_ID)
##              Df     AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## astar_null    3 1026.63 1035.9 -510.32  1020.63
## astar_linear  4  650.82  663.2 -321.41   642.82 377.81      1  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
anova(astar_null, astar_poly)
```

```
## Data: data
## Models:
## astar_null: a_star ~ 1 + (1 | Animal_ID)
## astar_poly: a_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
##             Df     AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## astar_null   3 1026.63 1035.91 -510.32  1020.63
## astar_poly   5  592.68  608.15 -291.34   582.68 437.95      2  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
anova(astar_linear, astar_poly)
```

```
## Data: data
## Models:
## astar_linear: a_star ~ O2Hb + (1 | Animal_ID)
## astar_poly: a_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
##              Df     AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## astar_linear  4 650.82 663.20 -321.41   642.82
## astar_poly    5 592.68 608.15 -291.34   582.68 60.137      1   8.85e-15 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Best fit model is the second-order polynomial (quadratic) model.

6.3   Explore the best fit model

```r
# Summary output
summary(astar_poly)
```

```
## Linear mixed model fit by maximum likelihood . t-tests use Satterthwaite's
##   method [lmerModLmerTest]
## Formula: a_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
##    Data: data
##
##      AIC      BIC   logLik deviance df.resid
##    592.7    608.2   -291.3    582.7      158
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.8260 -0.6074  0.0683  0.5903  2.6978
##
## Random effects:
##  Groups    Name        Variance Std.Dev.
##  Animal_ID (Intercept) 0.1578   0.3972
##  Residual              1.9902   1.4107
## Number of obs: 163, groups:  Animal_ID, 10
##
## Fixed effects:
##                Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)     38.9938     0.1725   8.7863 226.036  < 2e-16 ***
## poly(O2Hb, 2)1  68.1708     1.4682 162.9597  46.432  < 2e-16 ***
## poly(O2Hb, 2)2  12.3180     1.4444 162.1422   8.528 9.95e-15 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr) p(O2H,2)1
## ply(O2H,2)1 -0.009
## ply(O2H,2)2 -0.007 -0.010
```

```r
# Check model assumptions
plot_model(model = astar_poly,
           type = 'diag')[2:4]
```
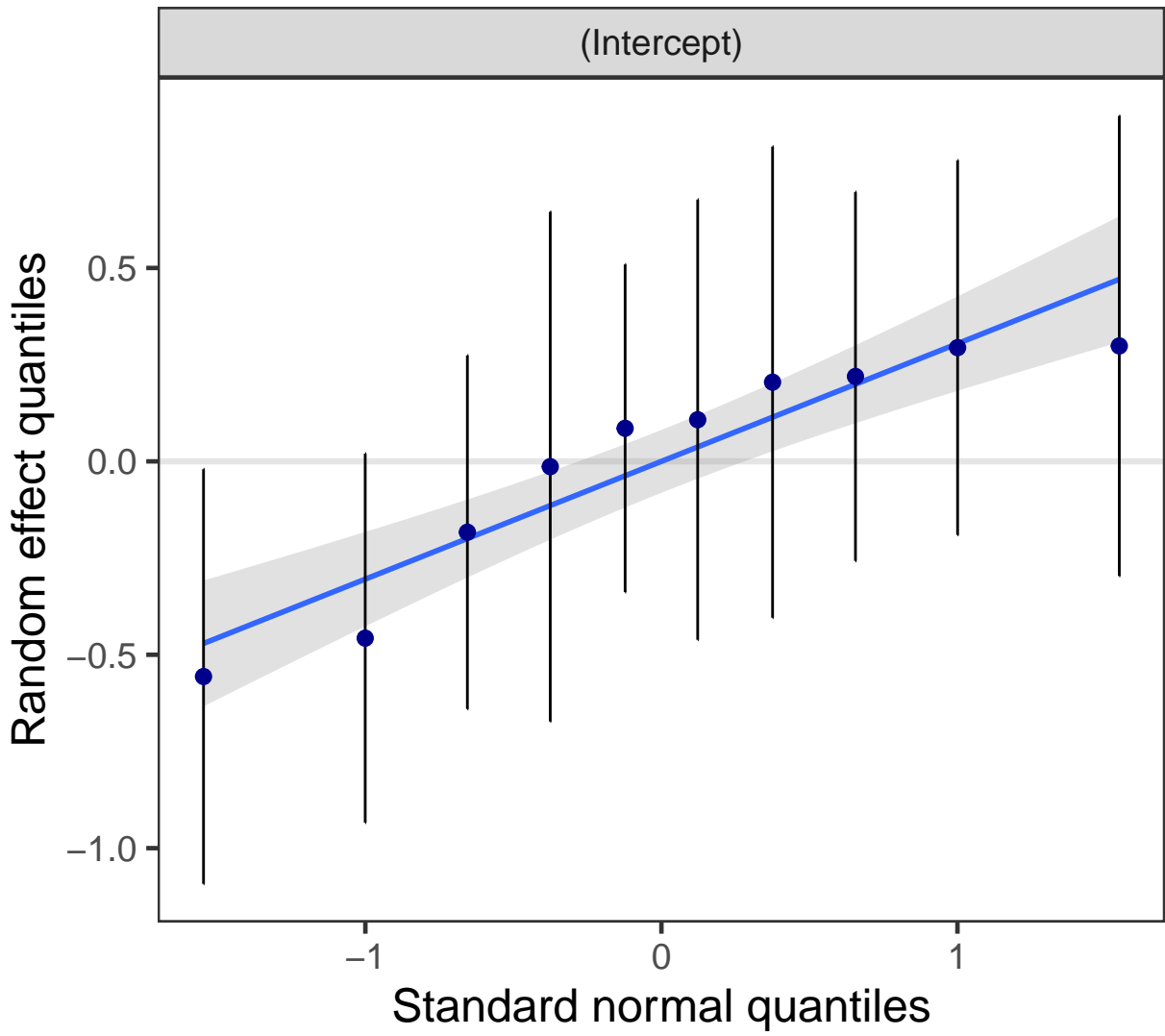
```
## [[1]]
## [[1]]$Animal_ID
```

```
##
##
## [[2]]
```

Non−normality of residuals

Distribution should look like normal curve

```
## 
## [[3]]
```
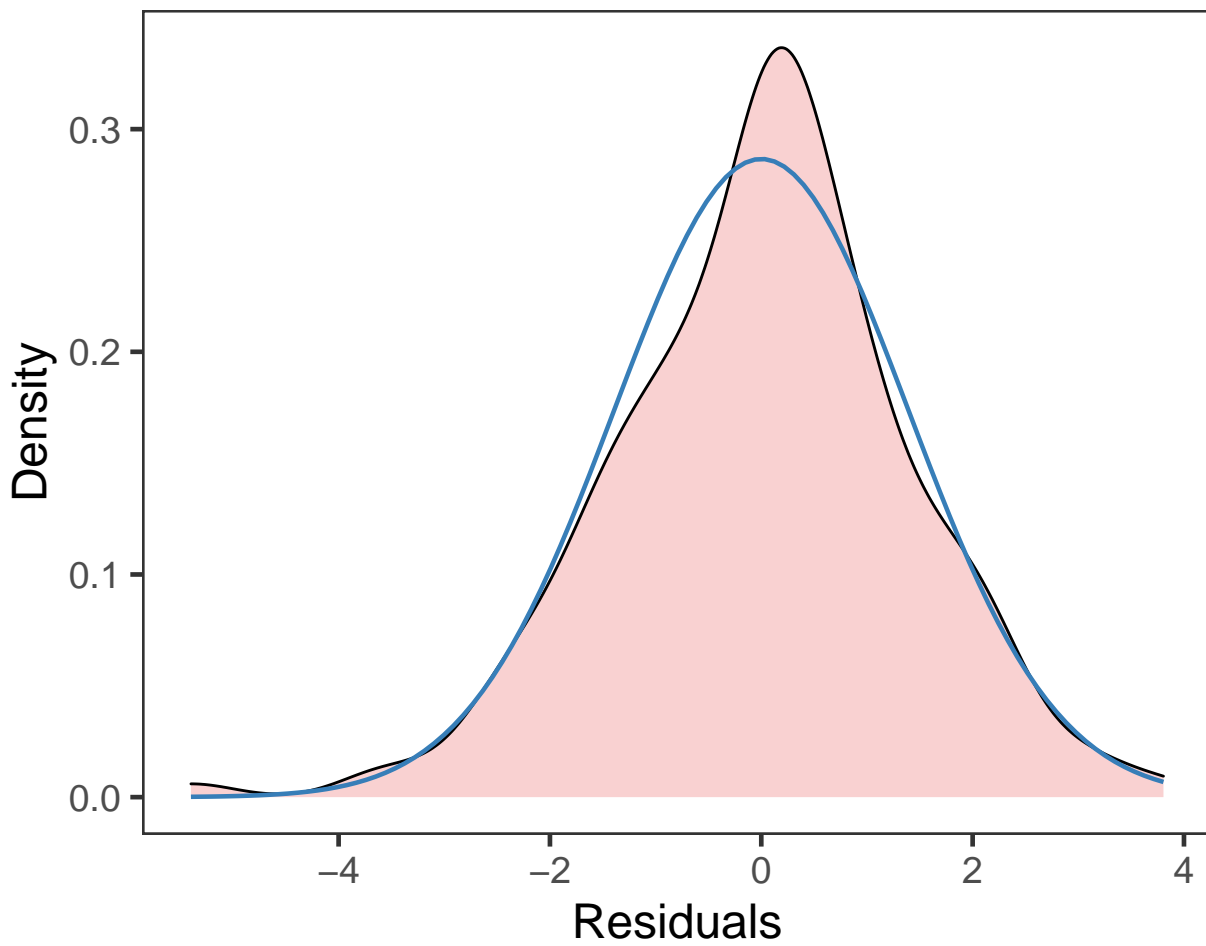
Homoscedasticity (constant variance of residuals)

Amount and distance of points scattered above/below line is equal
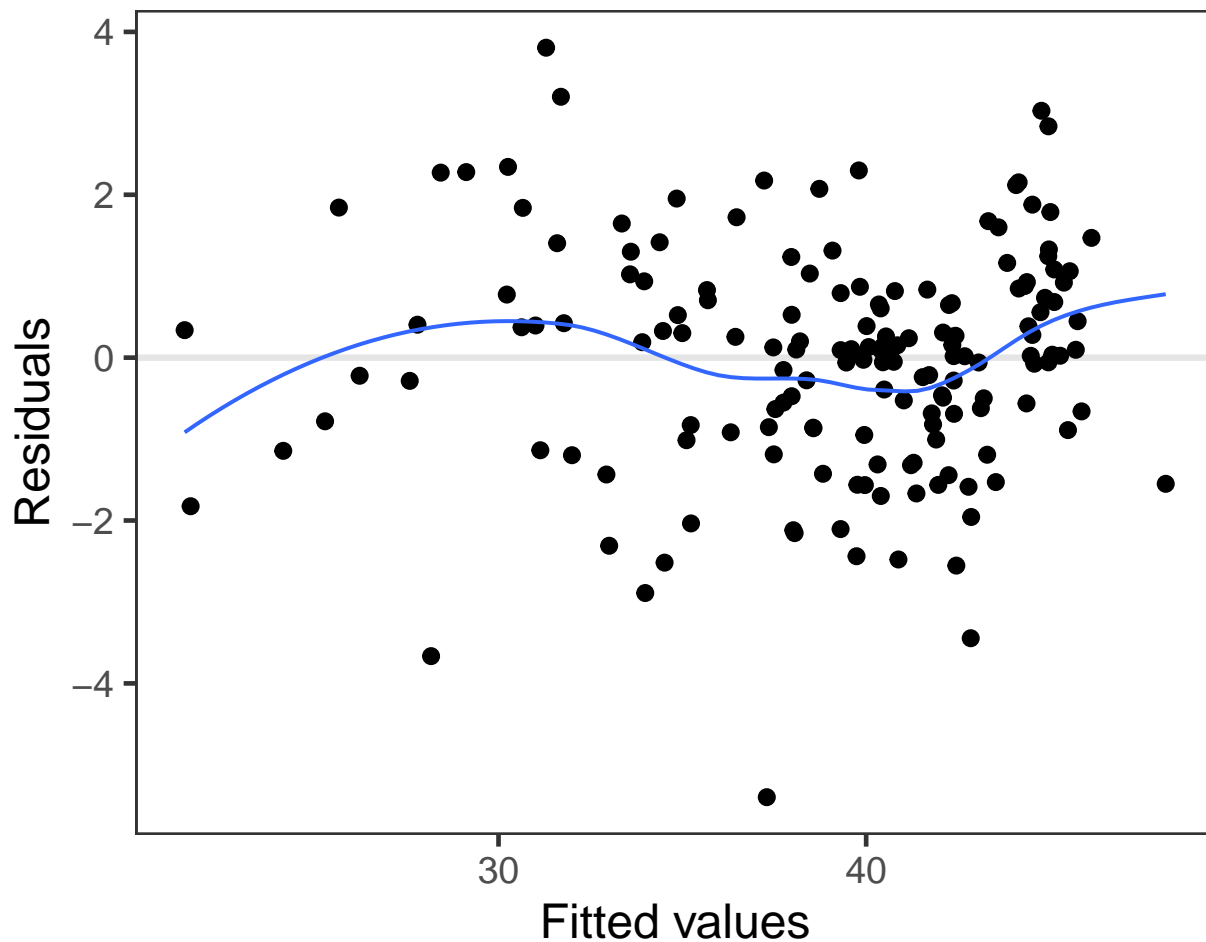

```r
# R-squared (based on likelihood ratio test)
r.squaredLR(astar_poly, null = astar_null)
```

```
## [1] 0.9319016
## attr(,"adj.r.squared")
## [1] 0.9336833
```

```r
# Root mean square error
RMSE.merMod(astar_poly)
```

```
## [1] 1.387596
```

6.4   Generate prediction intervals

```r
# Prediction interval
astar_lqmm <- lqmm(fixed = a_star ~ poly(O2Hb, 2),
                   random = ~ 1,
                   group = ~ Animal_ID,
                   tau = c(0.025, 0.976),
                   data = data)

astar_interval <- as.data.frame(predict(astar_lqmm, level = 0))

names(astar_interval) <- paste0('Q', c(2.5, 97.5))
```

```
astar_interval <- cbind(data, astar_interval)

# Numeric output
astar_interval %>%
    dplyr::select(O2Hb, a_star, Q2.5, Q97.5) %>%
    arrange(O2Hb) %>%
    unique(.) %>%
    kable(caption = 'a*: 95% prediction interval',
          col.names = c('FO2Hb (%)', 'a*', 'Q2.5', 'Q97.5'))
```

Table 5: a*: 95% prediction interval

	FO2Hb (%)	a*	Q2.5	Q97.5
1	15.7	21.8	18.71559	24.77128
2	18.7	19.8	18.98826	25.04958
3	35.4	23.0	21.46004	27.53400
4	40.7	24.5	22.58258	28.65393
5	41.8	27.5	22.83598	28.90638
6	44.6	26.0	23.51265	29.58003
7	49.7	27.3	24.86197	30.92156
8	51.0	24.5	25.23004	31.28717
9	51.9	30.7	25.49060	31.54591
10	52.3	28.2	25.60791	31.66238
11	54.2	31.4	26.17781	32.22806
12	58.4	32.6	27.51188	33.55134
13	58.6	31.0	27.57796	33.61686
14	59.5	31.0	27.87818	33.91449
15	59.6	32.5	27.91183	33.94784
16	60.0	31.4	28.04700	34.08183
17	60.6	30.0	28.25150	34.28451
18	61.7	33.0	28.63184	34.66142
19	62.2	34.9	28.80704	34.83501
20	62.2	35.1	28.80704	34.83501
21	64.3	32.2	29.55871	35.57963
22	64.9	30.8	29.77817	35.79698
23	65.4	31.5	29.96265	35.97968
24	65.8	30.7	30.11128	36.12685
25	66.5	35.0	30.37361	36.38660
26	67.1	34.6	30.60072	36.61146
27	67.7	34.9	30.82993	36.83836
28	68.1	34.9	30.98389	36.99077
29	68.4	31.1	31.09997	37.10567
30	68.5	34.1	31.13878	37.14409
31	69.4	34.8	31.49067	37.49237
32	69.7	35.8	31.60901	37.60949
33	69.7	32.0	31.60901	37.60949
34	70.6	35.4	31.96717	37.96392
35	70.8	36.8	32.04740	38.04331
36	70.9	35.3	32.08760	38.08309
37	71.0	34.1	32.12786	38.12292
38	71.3	33.2	32.24899	38.24277
39	71.5	34.4	32.33003	38.32296
40	72.6	36.4	32.77990	38.76803
41	73.1	36.5	32.98670	38.97261
42	74.4	35.4	33.53118	39.51117
43	74.7	36.7	33.65822	39.63682
44	75.4	38.2	33.95668	39.93198
45	76.2	39.4	34.30125	40.27273

	FO2Hb (%)	a*	Q2.5	Q97.5
46	76.6	37.6	34.47493	40.44447
47	76.8	36.5	34.56212	40.53068
48	76.8	36.3	34.56212	40.53068
49	76.9	36.9	34.60580	40.57387
50	77.4	37.6	34.82507	40.79067
51	77.4	37.2	34.82507	40.79067
52	77.9	31.9	35.04580	41.00890
53	77.9	38.5	35.04580	41.00890
54	77.9	37.5	35.04580	41.00890
55	78.4	39.2	35.26797	41.22854
56	78.9	35.9	35.49159	41.44961
57	79.1	38.1	35.58145	41.53844
58	79.3	38.4	35.67154	41.62749
59	79.4	40.8	35.71667	41.67210
60	79.8	35.9	35.89777	41.85111
61	79.9	38.2	35.94319	41.89601
62	80.3	37.4	36.12545	42.07616
63	80.6	40.4	36.26275	42.21187
64	80.7	39.5	36.30864	42.25722
65	80.7	37.7	36.30864	42.25722
66	80.9	37.7	36.40058	42.34810
67	81.2	39.4	36.53893	42.48483
68	81.7	37.2	36.77067	42.71386
69	81.9	42.1	36.86378	42.80587
70	82.1	38.2	36.95711	42.89810
71	82.2	40.7	37.00386	42.94431
72	82.5	40.1	37.14447	43.08326
73	82.5	39.4	37.14447	43.08326
74	82.5	39.0	37.14447	43.08326
75	82.9	39.7	37.33277	43.26932
76	83.0	39.7	37.37999	43.31598
77	83.1	38.4	37.42726	43.36270
78	83.2	37.3	37.47460	43.40947
79	83.2	40.4	37.47460	43.40947
80	83.4	38.7	37.56944	43.50318
81	83.8	39.9	37.75982	43.69129
82	83.9	41.0	37.80756	43.73846
83	84.0	41.0	37.85536	43.78569
84	84.1	40.2	37.90321	43.83297
85	84.2	41.6	37.95113	43.88031
86	84.2	40.1	37.95113	43.88031
87	84.3	40.8	37.99910	43.92771
88	84.6	41.4	38.14336	44.07025
89	84.6	39.0	38.14336	44.07025
90	84.6	40.5	38.14336	44.07025
91	84.7	40.6	38.19157	44.11787
92	84.9	40.4	38.28815	44.21329
93	85.0	38.4	38.33653	44.26109
94	85.1	40.7	38.38496	44.30894
95	85.3	40.7	38.48201	44.40481
96	85.3	40.5	38.48201	44.40481
97	85.5	41.0	38.57929	44.50092
98	85.7	39.9	38.67680	44.59725
99	86.0	39.7	38.82350	44.74217
100	86.1	41.0	38.87251	44.79059
101	86.3	41.1	38.97072	44.88760
102	86.5	42.4	39.06916	44.98484

	FO2Hb (%)	a*	Q2.5	Q97.5
103	86.5	40.9	39.06916	44.98484
104	86.6	40.0	39.11846	45.03354
105	86.6	42.5	39.11846	45.03354
106	86.7	41.5	39.16783	45.08231
107	86.9	41.3	39.26673	45.18000
108	87.0	42.5	39.31627	45.22893
109	87.1	41.7	39.36586	45.27792
110	87.2	42.9	39.41552	45.32697
111	87.2	40.4	39.41552	45.32697
112	87.4	43.0	39.51500	45.42523
113	87.4	41.6	39.51500	45.42523
114	87.5	42.4	39.56482	45.47445
115	87.5	42.1	39.56482	45.47445
116	87.6	42.7	39.61471	45.52372
117	87.6	39.9	39.61471	45.52372
118	88.0	39.4	39.81483	45.72138
119	88.1	42.7	39.86501	45.77094
120	88.2	41.6	39.91524	45.82056
121	88.4	40.9	40.01588	45.91996
122	88.5	40.8	40.06629	45.96974
123	88.5	41.2	40.06629	45.96974
124	88.5	42.6	40.06629	45.96974
125	88.7	42.7	40.16728	46.06948
126	88.7	42.5	40.16728	46.06948
127	88.8	43.0	40.21786	46.11944
128	89.1	45.0	40.36996	46.26965
129	89.3	42.1	40.47164	46.37007
130	89.9	45.2	40.77809	46.67271
131	90.1	45.0	40.88070	46.77404
132	90.6	46.2	41.13825	47.02837
133	90.7	46.3	41.18993	47.07940
134	91.0	42.0	41.34533	47.23285
135	91.8	45.2	41.76228	47.64455
136	91.9	45.3	41.81466	47.69627
137	92.0	45.0	41.86710	47.74805
138	92.1	45.6	41.91959	47.79988
139	92.1	47.8	41.91959	47.79988
141	92.1	44.5	41.91959	47.79988
142	92.2	46.8	41.97214	47.85177
143	92.3	46.3	42.02476	47.90371
144	92.4	43.8	42.07743	47.95571
145	92.5	44.8	42.13015	48.00777
146	92.5	44.9	42.13015	48.00777
147	92.6	45.3	42.18294	48.05989
148	92.7	44.8	42.23578	48.11206
149	92.7	46.4	42.23578	48.11206
150	92.7	45.1	42.23578	48.11206
151	92.8	44.5	42.28868	48.16429
152	92.8	45.8	42.28868	48.16429
153	92.8	46.2	42.28868	48.16429
154	92.9	46.3	42.34164	48.21658
155	93.0	46.2	42.39466	48.26892
156	93.1	44.6	42.44773	48.32132
157	93.1	45.3	42.44773	48.32132
158	93.2	46.6	42.50087	48.37378
159	93.5	45.8	42.66062	48.53150
160	93.6	46.2	42.71398	48.58418

	FO2Hb (%)	a*	Q2.5	Q97.5
161	93.8	45.2	42.82089	48.68972
162	94.3	47.6	43.08916	48.95457
163	98.1	46.6	45.17544	51.01384

```r
# Plot output
ggplot(data = astar_interval) +
    aes(x = O2Hb) +
    geom_point(data = data,
               aes(y = a_star,
                   x = O2Hb,
                   fill = Animal_ID),
               shape = 21,
               size = 3) +
    geom_smooth(data = astar_interval,
                aes(y = `Q2.5`),
                se = FALSE) +
    geom_smooth(data = astar_interval,
                aes(y = `Q97.5`),
                se = FALSE) +
    scale_fill_viridis_d(name = 'Animal') +
    labs(title = 'FO2Hb vs a*',
         subtitle = '95% prediction interval',
         y = 'a*',
         x = 'FO2Hb (%)')
```

FO2Hb vs a*

95% prediction interval
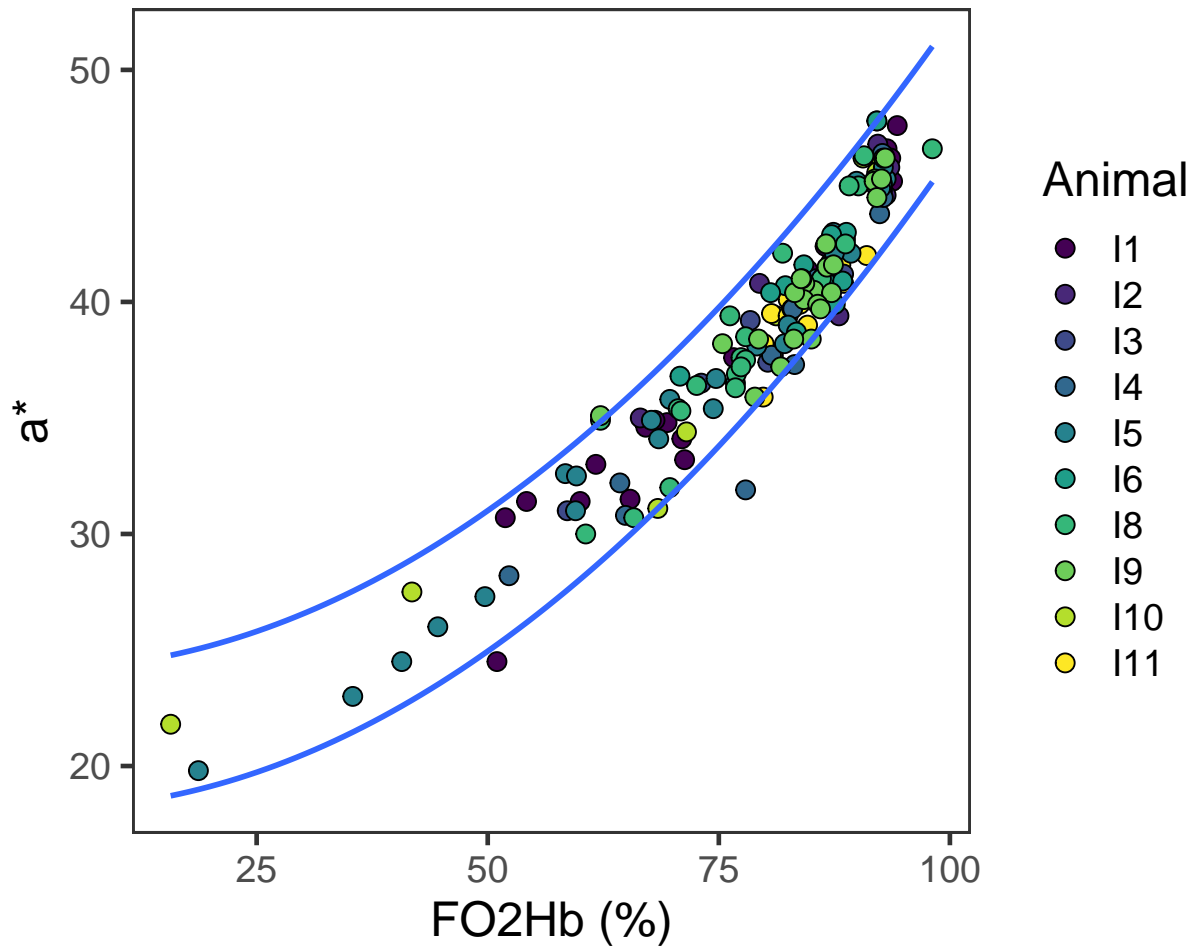
6.5 Generate predicted values

```
# New data
astar_grid <- expand.grid(O2Hb = c(15, 25, 35, 45, 55, 65, 75, 85, 95),
                          Animal_ID = 'I12')

# Generate predicted vs independent variable O2Hb
astar_predicted <- predict(astar_poly,
                           astar_grid,
                           allow.new.levels = TRUE)

# Tabulate
data.frame(O2Hb = astar_grid$O2Hb,
           astar = astar_predicted) %>%
    kable(caption = 'a* predicted values',
          col.names = c('FO2Hb (%)', 'a*'))
```

Table 6: a* predicted values

FO2Hb (%)	a*
15	21.19943
25	22.30015

FO2Hb (%)	a*
35	23.97979
45	26.23835
55	29.07583
65	32.49223
75	36.48756
85	41.06181
95	46.21497

7 b*

7.1 Exploratory plots
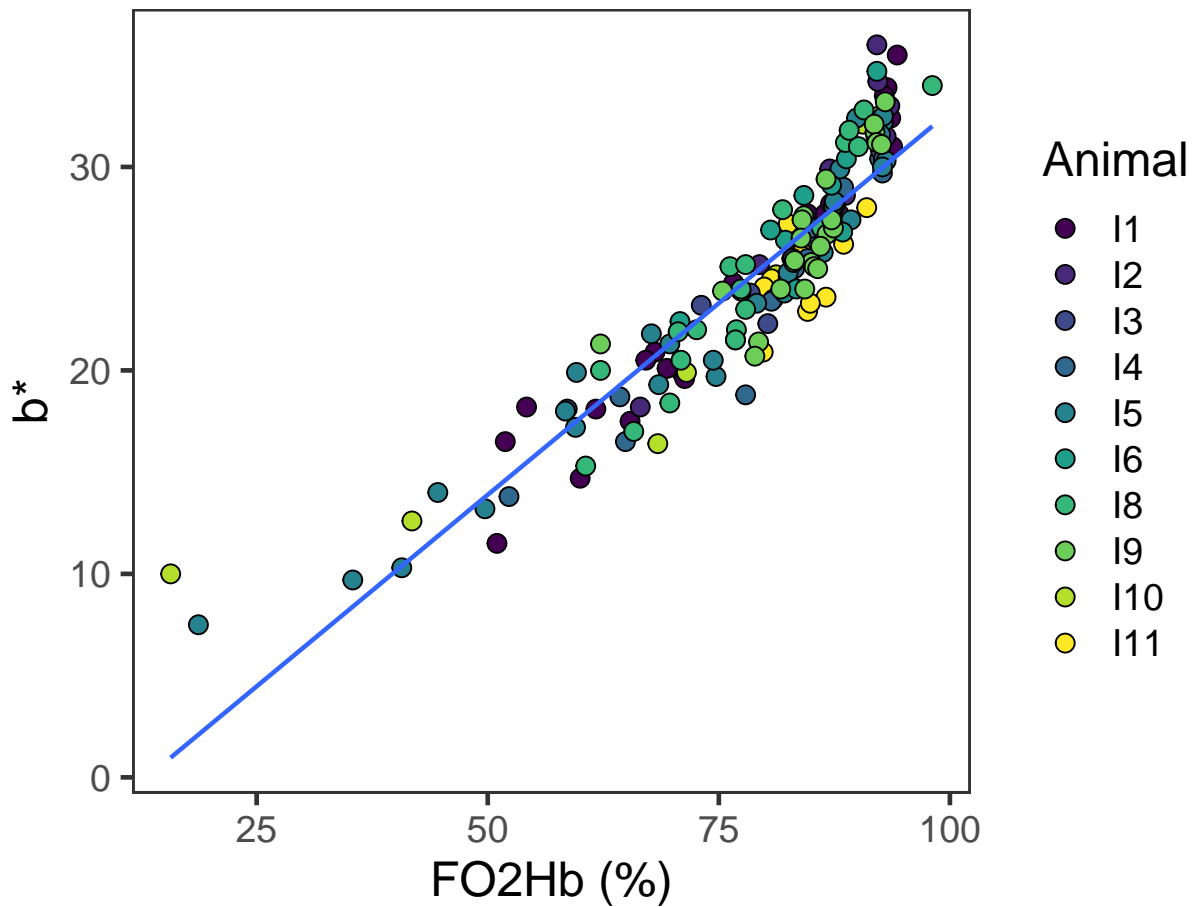
7.1.1 Linear model

```r
# Composite plot
ggplot(data = data) +
    aes(y = b_star,
        x = O2Hb,
        fill = Animal_ID) +
    geom_point(shape = 21,
               size = 3) +
    geom_smooth(se = FALSE,
                size = 0.8,
                method = 'lm',
                fill = 'blue') +
    scale_fill_viridis_d(name = 'Animal') +
    labs(title = 'FO2Hb vs b*',
         subtitle = 'All animals together\nRegression line is simple linear model',
         x = 'FO2Hb (%)',
         y = 'b*')
```

FO2Hb vs b*

All animals together
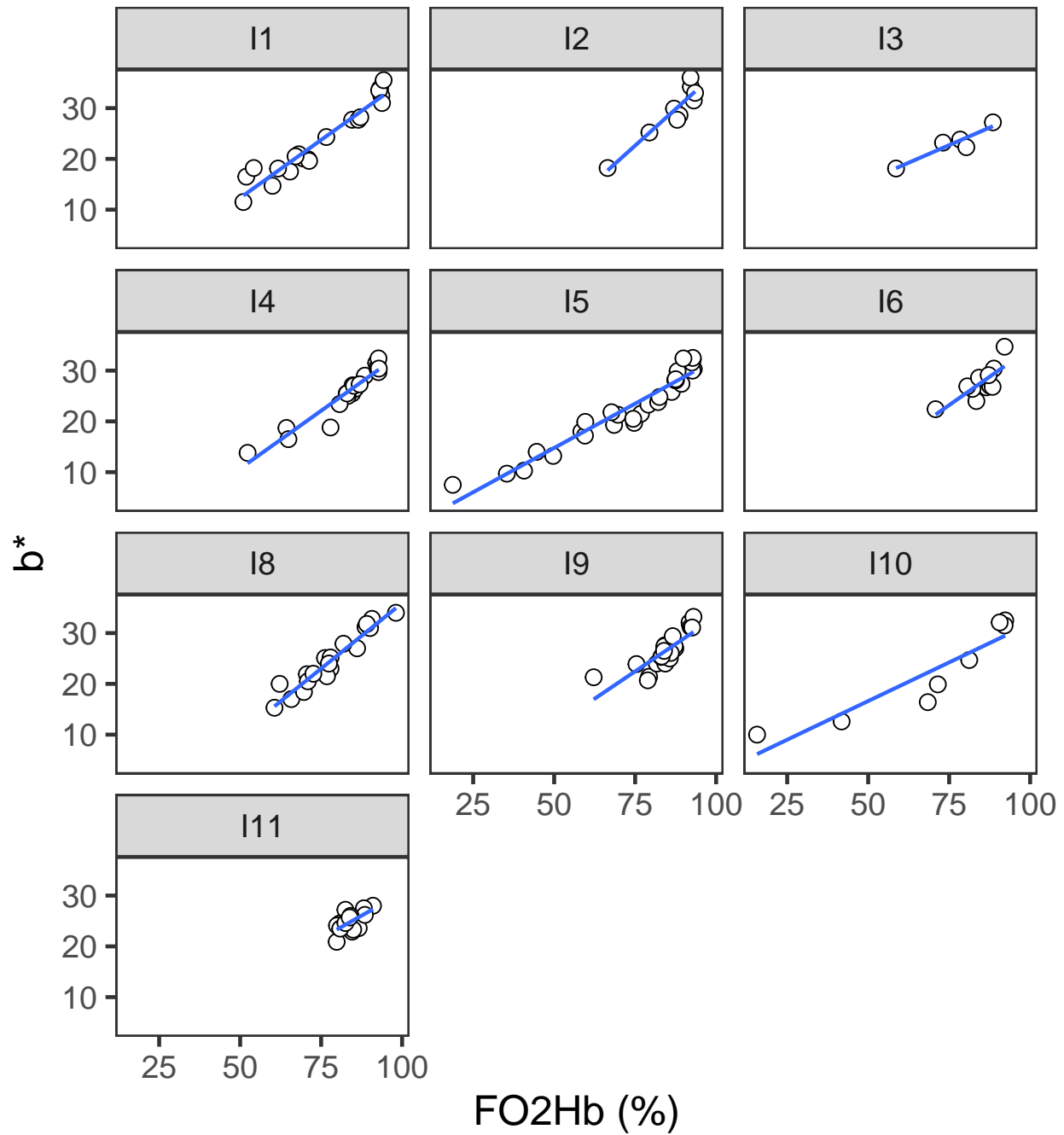Regression line is simple linear model


```r
# Small multiples
ggplot(data = data) +
    aes(y = b_star,
        x = O2Hb) +
    geom_point(shape = 21,
               size = 3,
               fill = '#FFFFFF') +
    geom_smooth(se = FALSE,
                size = 0.8,
                method = 'lm') +
    labs(title = 'FO2Hb vs b*',
         subtitle = 'Facetted by animal\nRegression line: linear',
         y = 'b*',
         x = 'FO2Hb (%)') +
    facet_wrap(~ Animal_ID,
               ncol = 3)
```

FO2Hb vs b*

Facetted by animal
Regression line: linear

7.1.2 Quadratic model
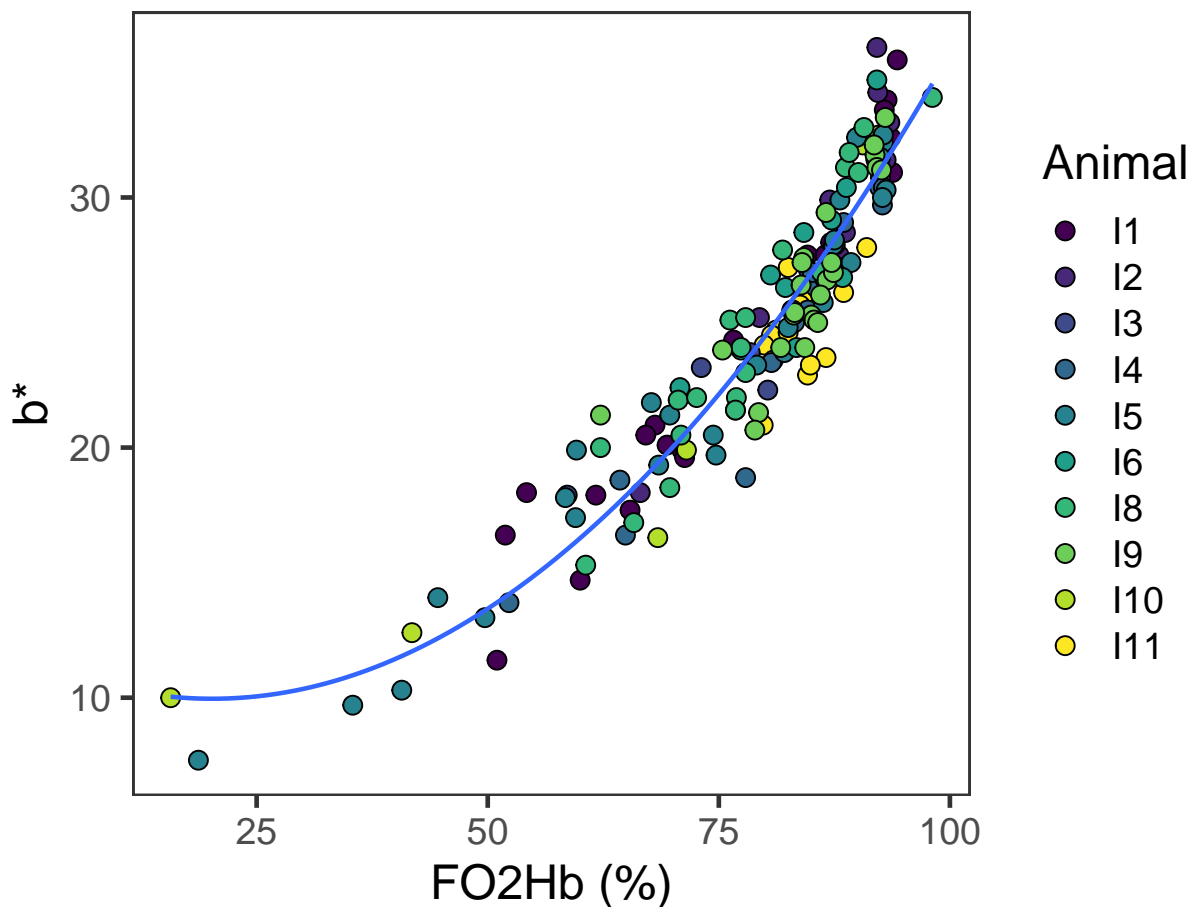
```r
# Composite plot
ggplot(data = data) +
    aes(y = b_star,
        x = O2Hb,
        fill = Animal_ID) +
    geom_point(shape = 21,
               size = 3) +
```

```
    geom_smooth(se = FALSE,
                size = 0.8,
                method = 'lm',
                fill = 'blue',
                formula = y ~ poly(x, 2)) +
    scale_fill_viridis_d(name = 'Animal') +
    labs(title = 'FO2Hb vs b*',
         subtitle = 'All animals together\nRegression line is a quadratic polynomial model',
         x = 'FO2Hb (%)',
         y = 'b*')
```

FO2Hb vs b*

All animals together
Regression line is a quadratic polynomial model


```
# Small multiples
ggplot(data = data) +
    aes(y = b_star,
        x = O2Hb) +
    geom_point(shape = 21,
               size = 3,
               fill = '#FFFFFF') +
    geom_smooth(se = FALSE,
                size = 0.8,
                colour = 'blue',
                method = 'lm',
                formula = y ~ poly(x, 2, raw = TRUE)) +
    labs(title = 'FO2Hb vs b*',
```

```
        subtitle = 'Facetted by animal\nRegression line: quadratic polynomial',
        y = 'b*',
        x = 'FO2Hb (%)') +
    facet_wrap(~ Animal_ID,
               ncol = 3)
```
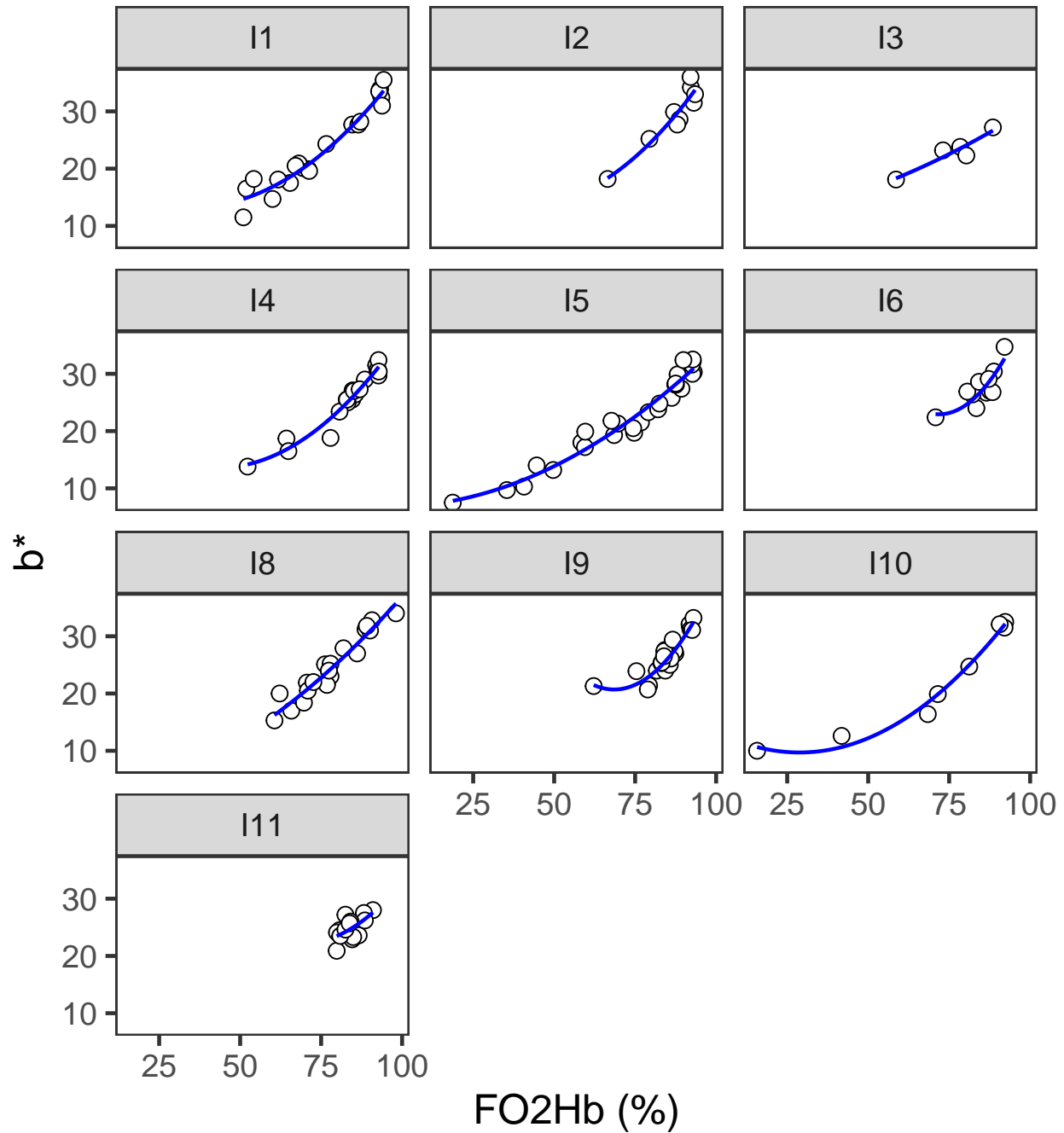
FO2Hb vs b*

Facetted by animal
Regression line: quadratic polynomial

7.2 Model selection

Model selection based on the outcome of likelihood ratio test of goodness of fit.

```r
# Null model
bstar_null <- lmer(b_star ~ 1 + (1 | Animal_ID),
                   data = data,
                   REML = FALSE)


## First-order polynomial (linear) model
bstar_linear <- lmer(b_star ~ O2Hb + (1 | Animal_ID),
                     data = data,
                     REML = FALSE)


## Second-order polynomial (quadratic) model
bstar_poly <- lmer(b_star ~ poly(O2Hb, 2) + (1 | Animal_ID),
                   data = data,
                   REML = FALSE)


# Compare model fits
anova(bstar_null, bstar_linear)
```

```
## Data: data
## Models:
## bstar_null: b_star ~ 1 + (1 | Animal_ID)
## bstar_linear: b_star ~ O2Hb + (1 | Animal_ID)
##              Df     AIC     BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## bstar_null    3 1036.44 1045.72 -515.22  1030.44
## bstar_linear  4  726.47  738.85 -359.24   718.47 311.97      1  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
anova(bstar_null, bstar_poly)
```

```
## Data: data
## Models:
## bstar_null: b_star ~ 1 + (1 | Animal_ID)
## bstar_poly: b_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
##              Df     AIC     BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## bstar_null    3 1036.44 1045.72 -515.22  1030.44
## bstar_poly    5  646.58  662.05 -318.29   636.58 393.86      2  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
anova(bstar_linear, bstar_poly)
```

```
## Data: data
## Models:
## bstar_linear: b_star ~ O2Hb + (1 | Animal_ID)
## bstar_poly: b_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
##              Df     AIC     BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## bstar_linear  4 726.47 738.85 -359.24   718.47
## bstar_poly    5 646.58 662.05 -318.29   636.58 81.889      1  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Best fit model is the second-order polynomial (quadratic) model.

## 7.3   Explore the best fit model

```r
# Summary output
summary(bstar_poly)
```

```
## Linear mixed model fit by maximum likelihood . t-tests use Satterthwaite's
##   method [lmerModLmerTest]
## Formula: b_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
##    Data: data
##
##      AIC      BIC   logLik deviance df.resid
##    646.6    662.1   -318.3    636.6      158
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.50851 -0.70799 -0.02569  0.68943  2.79398
##
## Random effects:
##  Groups    Name        Variance Std.Dev.
##  Animal_ID (Intercept) 0.445    0.6671
##  Residual              2.695    1.6416
## Number of obs: 163, groups:  Animal_ID, 10
##
## Fixed effects:
##                Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)     24.9897     0.2537   9.5248   98.49 1.19e-15 ***
## poly(O2Hb, 2)1  68.4258     1.7290 162.0411   39.58  < 2e-16 ***
## poly(O2Hb, 2)2  17.5254     1.6950 160.5744   10.34  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr) p(O2H,2)1
## ply(O2H,2)1 -0.007
## ply(O2H,2)2 -0.008 -0.014
```

```r
# Check model assumptions
plot_model(model = bstar_poly,
           type = 'diag')[2:4]
```
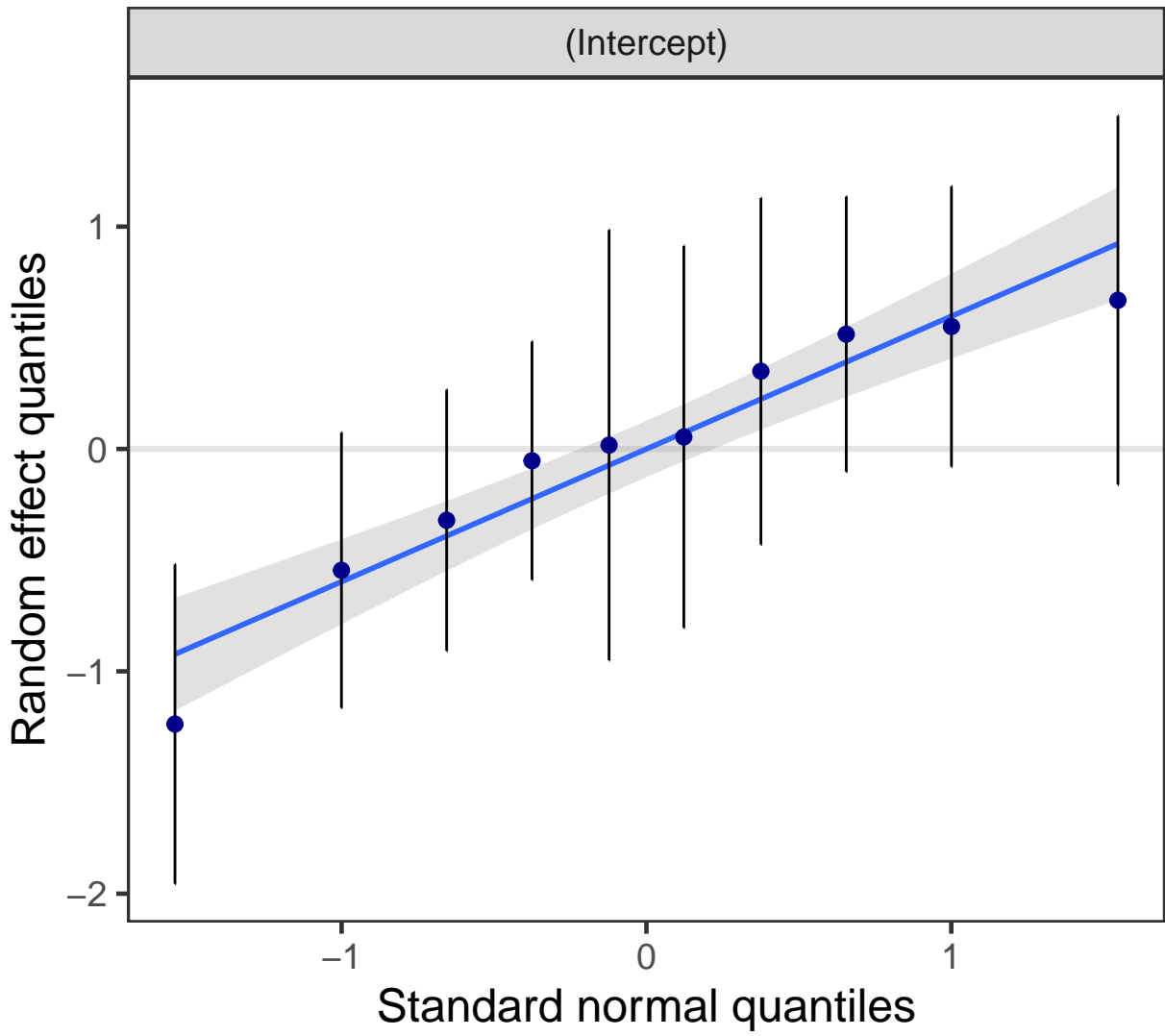
```
## [[1]]
## [[1]]$Animal_ID
```

```
##
##
## [[2]]
```

Non−normality of residuals

Distribution should look like normal curve


```
## 
## [[3]]
```
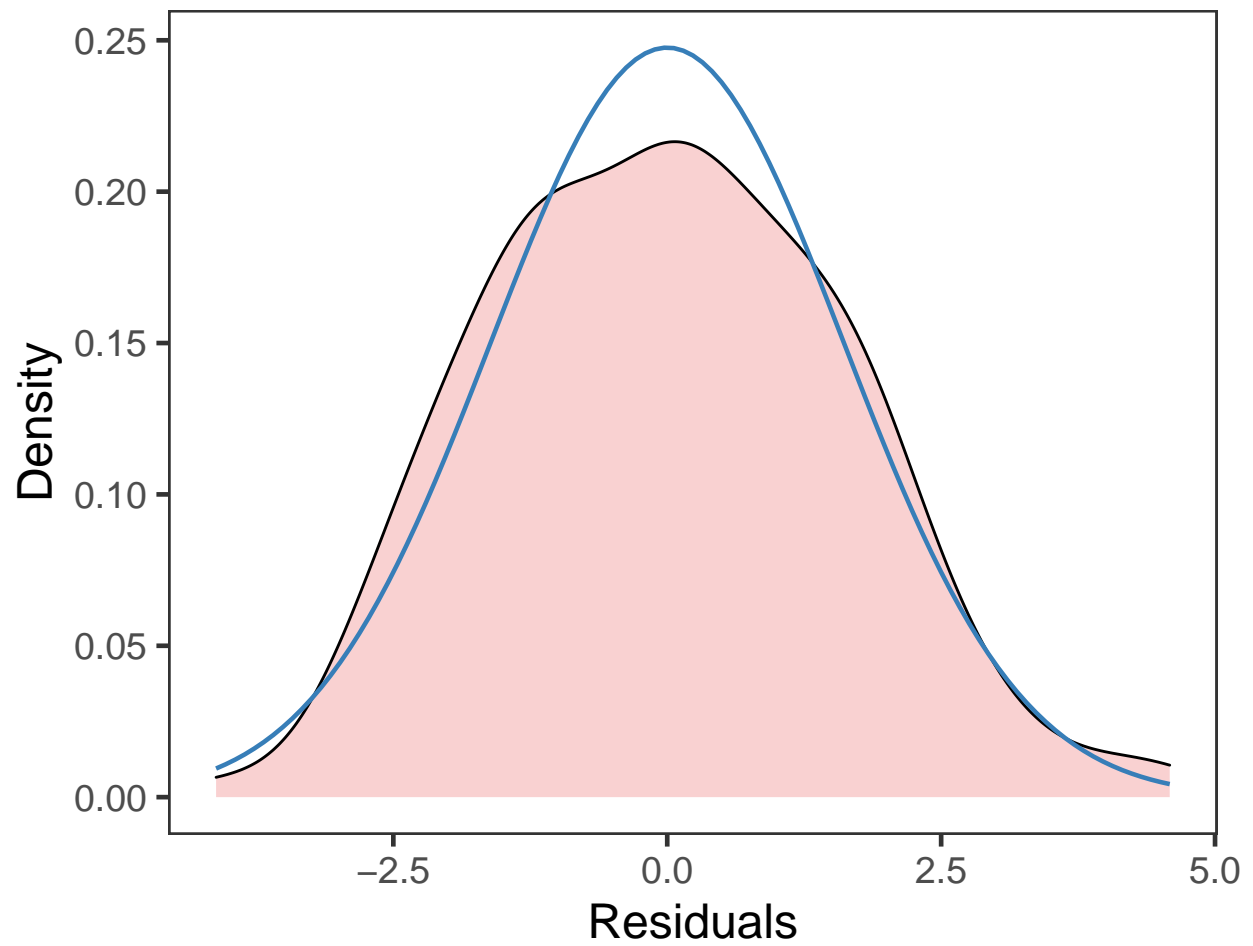
Homoscedasticity (constant variance of residuals)

Amount and distance of points scattered above/below line is equa
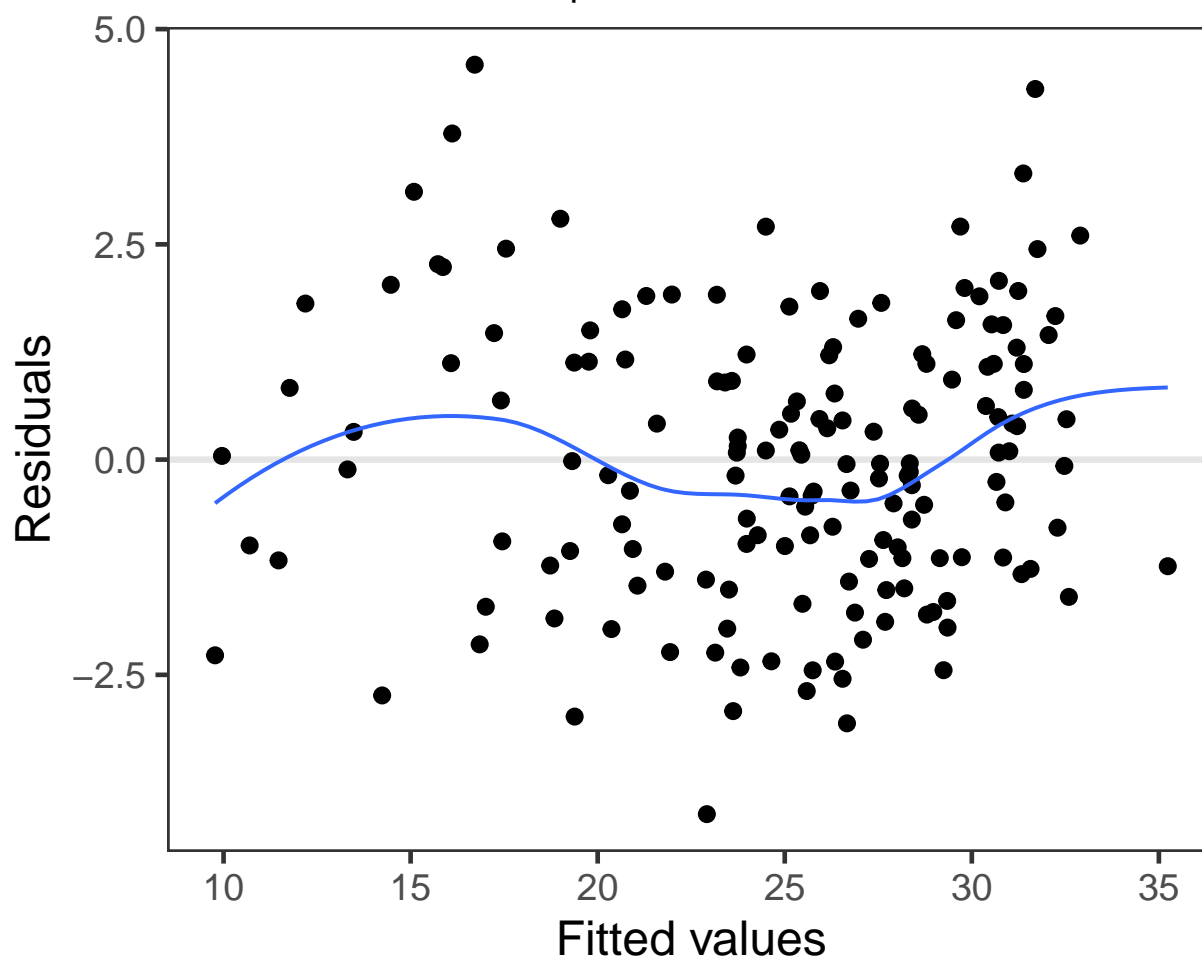

```r
# R-squared (based on likelihood ratio test)
r.squaredLR(bstar_poly, null = bstar_null)
```

```
## [1] 0.9107479
## attr(,"adj.r.squared")
## [1] 0.9123873
```

```r
# Root mean square error
RMSE.merMod(bstar_poly)
```

```
## [1] 1.606382
```

7.4   Generate prediction intervals

```r
# Prediction interval
bstar_lqmm <- lqmm(fixed = b_star ~ poly(O2Hb, 2),
                   random = ~ 1,
                   group = ~ Animal_ID,
                   tau = c(0.025, 0.976),
                   data = data)

bstar_interval <- as.data.frame(predict(bstar_lqmm, level = 0))

names(bstar_interval) <- paste0('Q', c(2.5, 97.5))
```

```r
bstar_interval <- cbind(data, bstar_interval)

# Numeric output
bstar_interval %>%
    dplyr::select(O2Hb, b_star, Q2.5, Q97.5) %>%
    arrange(O2Hb) %>%
    unique(.) %>%
    kable(caption = 'b*: 95% prediction interval',
          col.names = c('FO2Hb (%)', 'b*', 'Q2.5', 'Q97.5'))
```

Table 7: b*: 95% prediction interval

	FO2Hb (%)	b*	Q2.5	Q97.5
1	15.7	10.0	6.656783	13.69738
2	18.7	7.5	6.570083	13.62569
3	35.4	9.7	7.439507	14.55532
4	40.7	10.3	8.194624	15.32127
5	41.8	12.6	8.380278	15.50868
6	44.6	14.0	8.897732	16.02982
7	49.7	13.2	10.005816	17.14176
8	51.0	11.5	10.322461	17.45880
9	51.9	16.5	10.549815	17.68628
10	52.3	13.8	10.652998	17.78949
11	54.2	18.2	11.161077	18.29736
12	58.4	18.0	12.389489	19.52350
13	58.6	18.1	12.451601	19.58544
14	59.5	17.2	12.735175	19.86818
15	59.6	19.9	12.767094	19.90000
16	60.0	14.7	12.895593	20.02808
17	60.6	15.3	13.090807	20.22263
18	61.7	18.1	13.456385	20.58685
19	62.2	20.0	13.625844	20.75564
20	62.2	21.3	13.625844	20.75564
21	64.3	18.7	14.360012	21.48661
22	64.9	16.5	14.576432	21.70200
23	65.4	17.5	14.759042	21.88371
24	65.8	17.0	14.906610	22.03053
25	66.5	18.2	15.168019	22.29058
26	67.1	20.5	15.395288	22.51664
27	67.7	21.8	15.625517	22.74560
28	68.1	20.9	15.780646	22.89985
29	68.4	16.4	15.897857	23.01639
30	68.5	19.3	15.937091	23.05540
31	69.4	20.1	16.293900	23.41011
32	69.7	21.3	16.414316	23.52980
33	69.7	18.4	16.414316	23.52980
34	70.6	21.9	16.780002	23.89323
35	70.8	22.4	16.862170	23.97488
36	70.9	20.5	16.903377	24.01583
37	71.0	19.9	16.944667	24.05686
38	71.3	19.6	17.069028	24.18042
39	71.5	19.9	17.152346	24.26321
40	72.6	22.0	17.616474	24.72428
41	73.1	23.2	17.830729	24.93710
42	74.4	20.5	18.397409	25.49986
43	74.7	19.7	18.530154	25.63166
44	75.4	23.9	18.842769	25.94204
45	76.2	25.1	19.204975	26.30160

	FO2Hb (%)	b*	Q2.5	Q97.5
46	76.6	24.3	19.388051	26.48332
47	76.8	21.5	19.480082	26.57467
49	76.9	22.0	19.526221	26.62046
50	77.4	23.9	19.758148	26.85064
51	77.4	24.0	19.758148	26.85064
52	77.9	18.8	19.992130	27.08284
53	77.9	25.2	19.992130	27.08284
54	77.9	23.0	19.992130	27.08284
55	78.4	23.8	20.228166	27.31705
56	78.9	20.7	20.466258	27.55329
57	79.1	23.3	20.562069	27.64835
58	79.3	21.4	20.658210	27.74373
59	79.4	25.2	20.706404	27.79155
60	79.8	20.9	20.900000	27.98361
61	79.9	24.1	20.948605	28.03182
62	80.3	22.3	21.143845	28.22550
63	80.6	26.9	21.291139	28.37160
64	80.7	24.5	21.340401	28.42046
65	80.7	23.4	21.340401	28.42046
66	80.9	23.5	21.439172	28.51843
67	81.2	24.7	21.587944	28.66599
68	81.7	24.0	21.837543	28.91354
69	81.9	27.9	21.937958	29.01312
70	82.1	23.8	22.038701	29.11303
71	82.2	26.4	22.089196	29.16310
72	82.5	27.2	22.241175	29.31381
73	82.5	24.6	22.241175	29.31381
74	82.5	24.8	22.241175	29.31381
75	82.9	25.5	22.444964	29.51588
76	83.0	25.5	22.496116	29.56660
77	83.1	25.3	22.547351	29.61741
78	83.2	25.0	22.598668	29.66829
79	83.2	25.4	22.598668	29.66829
80	83.4	24.0	22.701549	29.77030
81	83.8	25.7	22.908296	29.97528
82	83.9	26.5	22.960189	30.02673
83	84.0	27.4	23.012163	30.07826
84	84.1	26.0	23.064220	30.12986
85	84.2	28.6	23.116359	30.18155
86	84.2	27.6	23.116359	30.18155
87	84.3	24.0	23.168580	30.23333
88	84.6	27.7	23.325737	30.38912
89	84.6	22.9	23.325737	30.38912
90	84.6	25.5	23.325737	30.38912
91	84.7	27.1	23.378287	30.44122
92	84.9	23.3	23.483633	30.54565
93	85.0	25.3	23.536429	30.59798
94	85.1	27.0	23.589308	30.65040
95	85.3	26.6	23.695312	30.75548
96	85.3	25.1	23.695312	30.75548
97	85.5	26.4	23.801645	30.86088
98	85.7	25.0	23.908306	30.96660
99	86.0	26.1	24.068915	31.12579
100	86.1	27.0	24.122616	31.17901
101	86.3	25.8	24.230264	31.28571
102	86.5	27.7	24.338240	31.39272
103	86.5	26.7	24.338240	31.39272

	FO2Hb (%)	b*	Q2.5	Q97.5
104	86.6	23.6	24.392352	31.44635
105	86.6	29.4	24.392352	31.44635
106	86.7	26.7	24.446546	31.50006
107	86.9	27.3	24.555180	31.60772
108	87.0	29.9	24.609620	31.66167
109	87.1	28.2	24.664143	31.71571
110	87.2	29.1	24.718748	31.76982
111	87.2	27.4	24.718748	31.76982
112	87.4	28.1	24.828204	31.87829
113	87.4	27.0	24.828204	31.87829
114	87.5	28.2	24.883055	31.93264
115	87.5	28.3	24.883055	31.93264
116	87.6	28.1	24.937989	31.98708
117	87.6	27.0	24.937989	31.98708
118	88.0	27.7	25.158545	32.20563
119	88.1	29.9	25.213890	32.26047
120	88.2	27.5	25.269316	32.31540
121	88.4	26.8	25.380416	32.42548
122	88.5	26.2	25.436090	32.48064
123	88.5	27.2	25.436090	32.48064
124	88.5	29.0	25.436090	32.48064
125	88.7	28.6	25.547683	32.59121
126	88.7	31.2	25.547683	32.59121
127	88.8	30.4	25.603603	32.64662
128	89.1	31.8	25.771856	32.81332
129	89.3	27.4	25.884435	32.92486
130	89.9	32.4	26.224146	33.26141
131	90.1	31.0	26.338041	33.37425
132	90.6	32.1	26.624216	33.65774
133	90.7	32.8	26.681697	33.71468
134	91.0	28.0	26.854635	33.88598
135	91.8	32.1	27.319419	34.34635
136	91.9	31.7	27.377887	34.40426
137	92.0	31.5	27.436438	34.46226
138	92.1	31.5	27.495070	34.52033
139	92.1	36.0	27.495070	34.52033
140	92.1	34.7	27.495070	34.52033
141	92.1	31.2	27.495070	34.52033
142	92.2	34.2	27.553784	34.57848
143	92.3	32.5	27.612581	34.63671
144	92.4	30.4	27.671460	34.69503
145	92.5	30.8	27.730421	34.75342
146	92.5	31.6	27.730421	34.75342
147	92.6	31.1	27.789464	34.81190
148	92.7	29.7	27.848590	34.87045
149	92.7	32.4	27.848590	34.87045
150	92.7	30.0	27.848590	34.87045
151	92.8	30.4	27.907797	34.92909
152	92.8	32.2	27.907797	34.92909
153	92.8	32.5	27.907797	34.92909
154	92.9	33.5	27.967087	34.98781
155	93.0	33.2	28.026459	35.04660
156	93.1	31.5	28.085913	35.10548
157	93.1	30.3	28.085913	35.10548
158	93.2	33.9	28.145450	35.16444
159	93.5	33.0	28.324552	35.34181
160	93.6	32.4	28.384417	35.40109

	FO2Hb (%)	b*	Q2.5	Q97.5
161	93.8	31.0	28.504394	35.51990
162	94.3	35.5	28.805775	35.81834
163	98.1	34.0	31.163422	38.15244

```r
# Plot output
ggplot(data = bstar_interval) +
    aes(x = O2Hb) +
    geom_point(data = data,
               aes(y = b_star,
                   x = O2Hb,
                   fill = Animal_ID),
               shape = 21,
               size = 3) +
    geom_smooth(data = bstar_interval,
                aes(y = `Q2.5`),
                se = FALSE) +
    geom_smooth(data = bstar_interval,
                aes(y = `Q97.5`),
                se = FALSE) +
    scale_fill_viridis_d(name = 'Animal') +
    labs(title = 'FO2Hb vs b*',
         subtitle = '95% prediction interval',
         y = 'b*',
         x = 'FO2Hb (%)')
```

FO2Hb vs b*
95% prediction interval
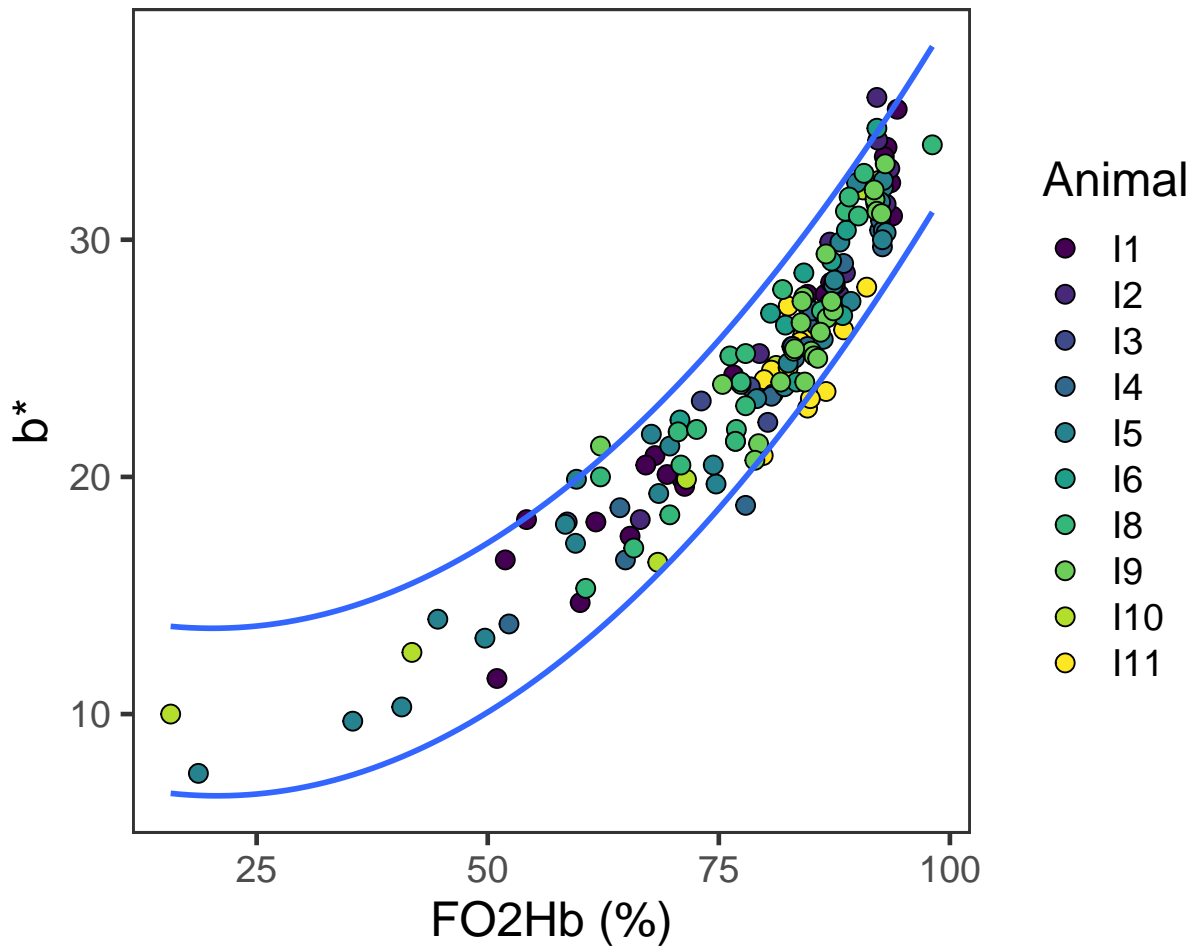
7.5 Generate predicted values

```r
# New data
bstar_grid <- expand.grid(O2Hb = c(15, 25, 35, 45, 55, 65, 75, 85, 95),
                          Animal_ID = 'I12')

# Generate predicted vs independent variable O2Hb
bstar_predicted <- predict(bstar_poly,
                           bstar_grid,
                           allow.new.levels = TRUE)

# Tabulate
data.frame(O2Hb = bstar_grid$O2Hb,
           bstar = bstar_predicted) %>%
   kable(caption = 'b* predicted values',
         col.names = c('FO2Hb (%)', 'b*'))
```

Table 8: b* predicted values

FO2Hb (%)	b*
15	9.932111
25	9.904795

FO2Hb (%)	b*
35	10.701137
45	12.321137
55	14.764796
65	18.032113
75	22.123088
85	27.037722
95	32.776014

8 Code for publication plots

```r
library(patchwork)

lplot <- ggplot(data = lstar_interval) +
    aes(x = O2Hb) +
    geom_point(data = data,
               aes(y = l_star,
                   x = O2Hb,
                   fill = Animal_ID),
               shape = 21,
               size = 3) +
    geom_smooth(data = lstar_interval,
                aes(y = `Q2.5`),
                se = FALSE) +
    geom_smooth(data = lstar_interval,
                aes(y = `Q97.5`),
                se = FALSE) +
    scale_fill_viridis_d(name = 'Animal') +
    labs(y = 'L*') +
    theme(axis.title.x = element_blank())

aplot <- ggplot(data = astar_interval) +
    aes(x = O2Hb) +
    geom_point(data = data,
               aes(y = a_star,
                   x = O2Hb,
                   fill = Animal_ID),
               shape = 21,
               size = 3) +
    geom_smooth(data = astar_interval,
                aes(y = `Q2.5`),
                se = FALSE) +
    geom_smooth(data = astar_interval,
                aes(y = `Q97.5`),
                se = FALSE) +
    scale_fill_viridis_d(name = 'Animal') +
    labs(y = 'a*') +
    theme(axis.title.x = element_blank(),
          legend.position = 'none')

bplot <- ggplot(data = bstar_interval) +
    aes(x = O2Hb) +
    geom_point(data = data,
```

```r
            aes(y = b_star,
                x = O2Hb,
                fill = Animal_ID),
            shape = 21,
            size = 3) +
    geom_smooth(data = bstar_interval,
                aes(y = `Q2.5`),
                se = FALSE) +
    geom_smooth(data = bstar_interval,
                aes(y = `Q97.5`),
                se = FALSE) +
    scale_fill_viridis_d(name = 'Animal') +
    labs(y = 'b*',
         x = expression('FO'[2]*'Hb (%)')) +
    theme(legend.position = 'none')

# Construct plot
pub_plot <- lplot + aplot + bplot +
    plot_layout(ncol = 1) +
    plot_annotation(tag_levels = 'A')

ggsave(filename = 'figures/figure_1b.png',
       plot = pub_plot,
       height = 11,
       width = 6)
```

---

9 Session Information

```r
sessionInfo()
```

```
## R version 3.6.3 (2020-02-29)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Catalina 10.15.4
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] knitr_1.28        MuMIn_1.43.15     lqmm_1.5.5        sjPlot_2.8.3
##  [5] merTools_0.5.0    arm_1.10-1        MASS_7.3-51.5     lmerTest_3.1-1
##  [9] lme4_1.1-21       Matrix_1.2-18     skimr_2.1         magrittr_1.5
## [13] forcats_0.5.0     stringr_1.4.0     dplyr_0.8.5       purrr_0.3.3
## [17] readr_1.3.1       tidyr_1.0.2       tibble_3.0.0      ggplot2_3.3.0.9000
## [21] tidyverse_1.3.0
##
## loaded via a namespace (and not attached):
##  [1] TH.data_1.0-10    minqa_1.2.4       colorspace_1.4-1
##  [4] ellipsis_0.3.0    sjlabelled_1.1.3  estimability_1.3
```

```
##  [7]  parameters_0.6.0      base64enc_0.1-3    fs_1.3.1
## [10]  rstudioapi_0.11       glmmTMB_1.0.1      farver_2.0.3
## [13]  fansi_0.4.1           mvtnorm_1.1-0      lubridate_1.7.4
## [16]  xml2_1.3.0            codetools_0.2-16   splines_3.6.3
## [19]  sjmisc_2.8.4          jsonlite_1.6.1     nloptr_1.2.2.1
## [22]  ggeffects_0.14.2      broom_0.5.5        dbplyr_1.4.2
## [25]  effectsize_0.2.0      shiny_1.4.0.2      compiler_3.6.3
## [28]  httr_1.4.1            sjstats_0.17.9     emmeans_1.4.5
## [31]  backports_1.1.5       assertthat_0.2.1   fastmap_1.0.1
## [34]  cli_2.0.2            later_1.0.0         htmltools_0.4.0
## [37]  tools_3.6.3           SparseGrid_0.8.2   coda_0.19-3
## [40]  gtable_0.3.0          glue_1.3.2         Rcpp_1.0.4
## [43]  cellranger_1.1.0      vctrs_0.2.4        nlme_3.1-145
## [46]  iterators_1.0.12      insight_0.8.2      xfun_0.12
## [49]  rvest_0.3.5           mime_0.9           lifecycle_0.2.0
## [52]  zoo_1.8-7             scales_1.1.0       hms_0.5.3
## [55]  promises_1.1.0        sandwich_2.5-1     TMB_1.7.16
## [58]  RColorBrewer_1.1-2    yaml_2.2.1         stringi_1.4.6
## [61]  highr_0.8             bayestestR_0.5.3   foreach_1.5.0
## [64]  blme_1.0-4            boot_1.3-24        repr_1.1.0
## [67]  rlang_0.4.5           pkgconfig_2.0.3    evaluate_0.14
## [70]  lattice_0.20-38       labeling_0.3       tidyselect_1.0.0
## [73]  R6_2.4.1              generics_0.0.2     multcomp_1.4-12
## [76]  DBI_1.1.0             mgcv_1.8-31        pillar_1.4.3
## [79]  haven_2.2.0           withr_2.1.2        survival_3.1-11
## [82]  abind_1.4-5           performance_0.4.5  modelr_0.1.6
## [85]  crayon_1.3.4          utf8_1.1.4         rmarkdown_2.1
## [88]  grid_3.6.3            readxl_1.3.1       reprex_0.3.0
## [91]  digest_0.6.25         xtable_1.8-4       httpuv_1.5.2
## [94]  numDeriv_2016.8-1.1   stats4_3.6.3       munsell_0.5.0
## [97]  viridisLite_0.3.0
```