SUPPORTING INFORMATION

PROTEOMICS
Research article

PROTEOMICS-BASED SCORING OF CELLULAR RESPONSE TO STIMULI FOR IMPROVED CHARACTERIZATION OF SIGNALING PATHWAY ACTIVITY

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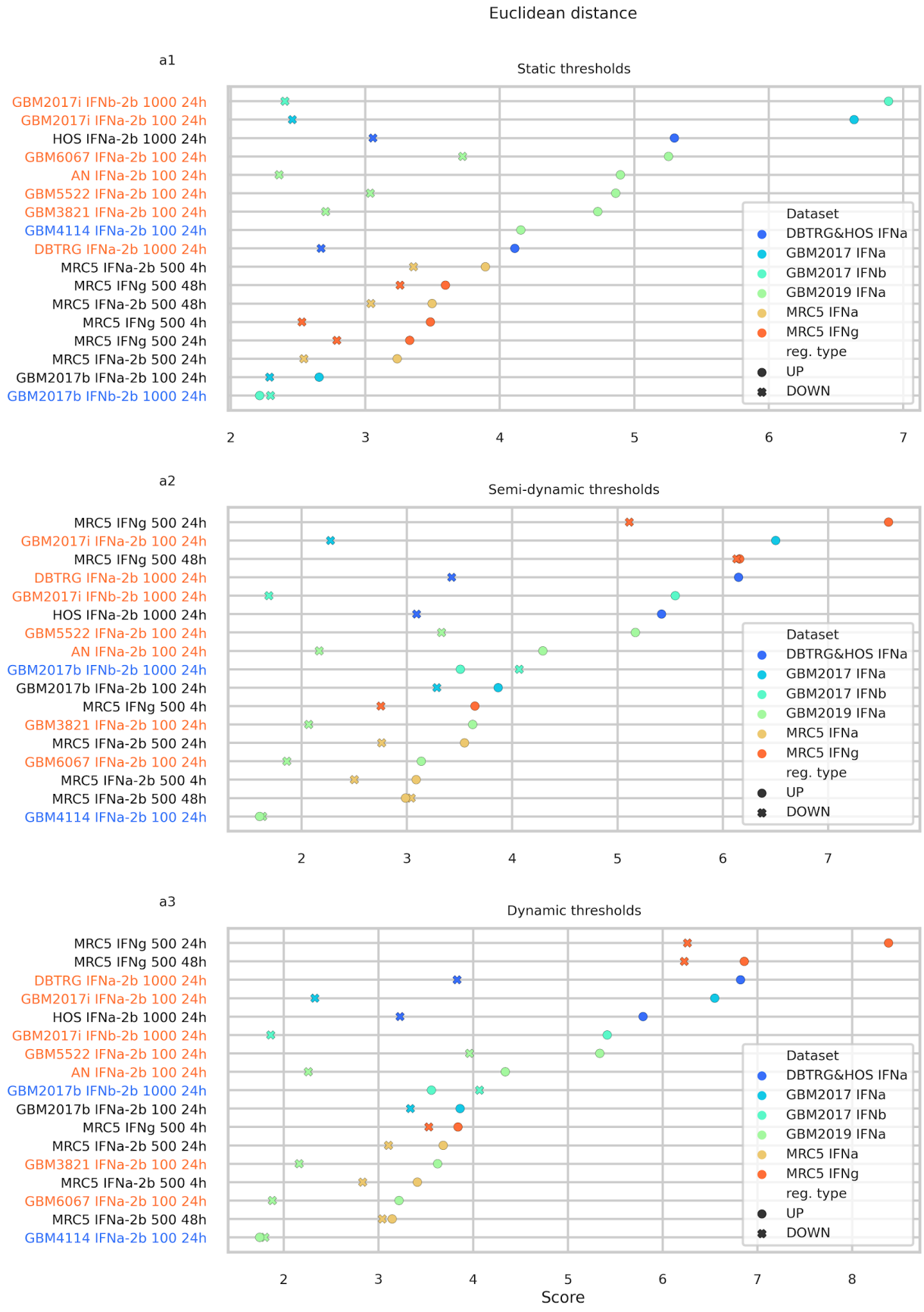
Tables S1-S7 *are provided as separate .xlsx files at https://github.com/kazakova/Metrics.*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AN IFNa-2b 100 24h
GBM5522 IFNa-2b 100 24h
GBM3821 IFNa-2b 100 24h
GBM4114 IFNa-2b 100 24h
DBTRG IFNa-2b 1000 24h
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MRC5 IFNg
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2
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a2
Semi-dynamic thresholds
MRC5 IFNg 500 24h
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MRC5 IFNg 500 48h
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GBM2017i IFNb-2b 1000 24h
HOS IFNa-2b 1000 24h
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MRC5 IFNa-2b 500 48h
GBM4114 IFNa-2b 100 24h
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GBM2017 IFNb
GBM2019 IFNa
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MRC5 IFNg
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UP
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a3
Dynamic thresholds
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GBM6067 IFNa-2b 100 24h
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GBM4114 IFNa-2b 100 24h
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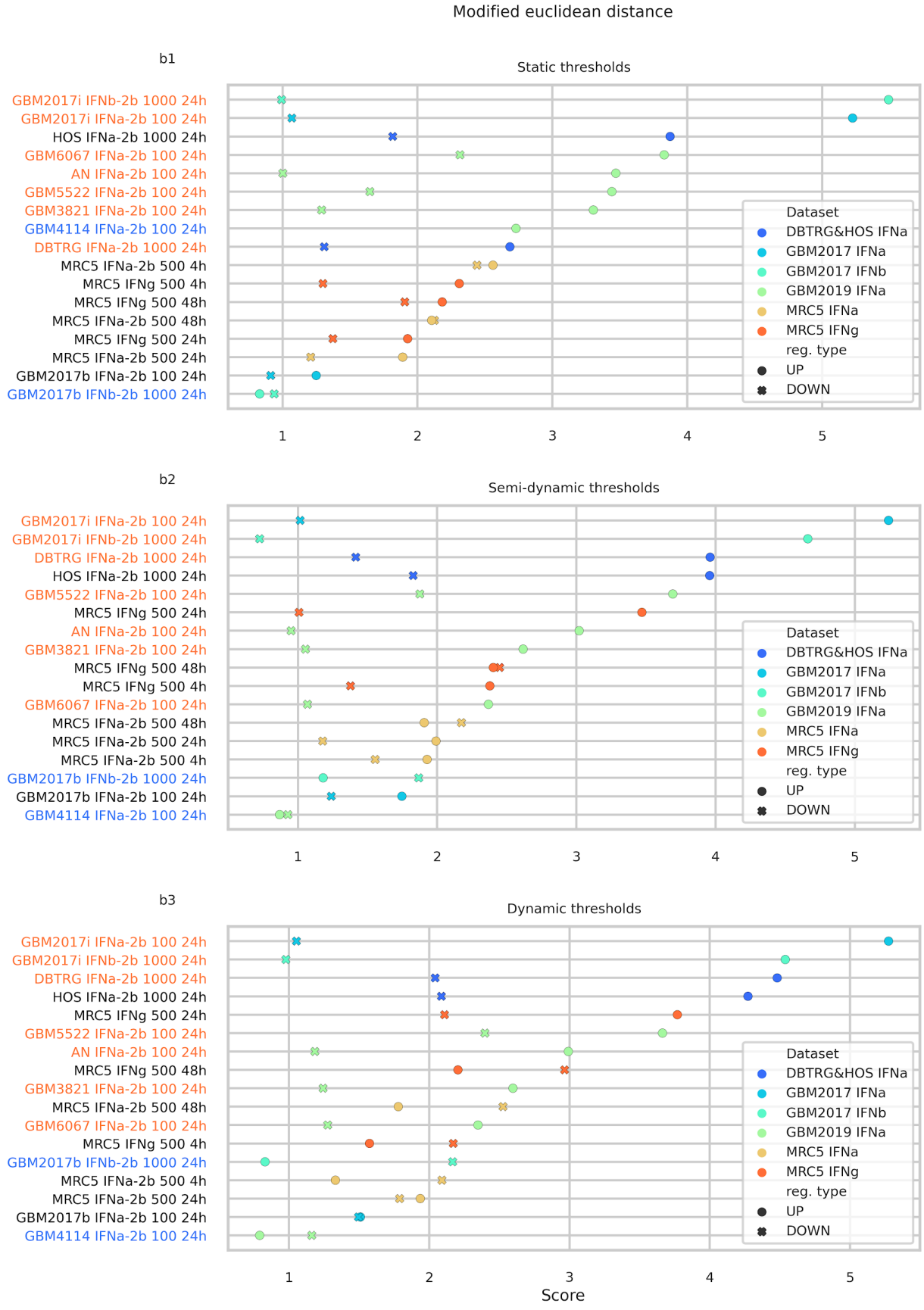
7
8
Score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GBM3821 IFNa-2b 100 24h
GBM4114 IFNa-2b 100 24h
DBTRG IFNa-2b 1000 24h
MRC5 IFNa-2b 500 4h
MRC5 IFNg 500 4h
MRC5 IFNg 500 48h
MRC5 IFNa-2b 500 48h
MRC5 IFNg 500 24h
MRC5 IFNa-2b 500 24h
GBM2017b IFNa-2b 100 24h
GBM2017b IFNb-2b 1000 24h
Dataset
DBTRG&HOS IFNa
GBM2017 IFNa
GBM2017 IFNb
GBM2019 IFNa
MRC5 IFNa
MRC5 IFNg
reg. type
UP
DOWN
1
2
3
4
5
b2
Semi-dynamic thresholds
GBM2017i IFNa-2b 100 24h
GBM2017i IFNb-2b 1000 24h
DBTRG IFNa-2b 1000 24h
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GBM5522 IFNa-2b 100 24h
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MRC5 IFNa-2b 500 48h
MRC5 IFNa-2b 500 24h
MRC5 IFNa-2b 500 4h
GBM2017b IFNb-2b 1000 24h
GBM2017b IFNa-2b 100 24h
GBM4114 IFNa-2b 100 24h
Dataset
DBTRG&HOS IFNa
GBM2017 IFNa
GBM2017 IFNb
GBM2019 IFNa
MRC5 IFNa
MRC5 IFNg
reg. type
UP
DOWN
1
2
3
4
5
b3
Dynamic thresholds
GBM2017i IFNa-2b 100 24h
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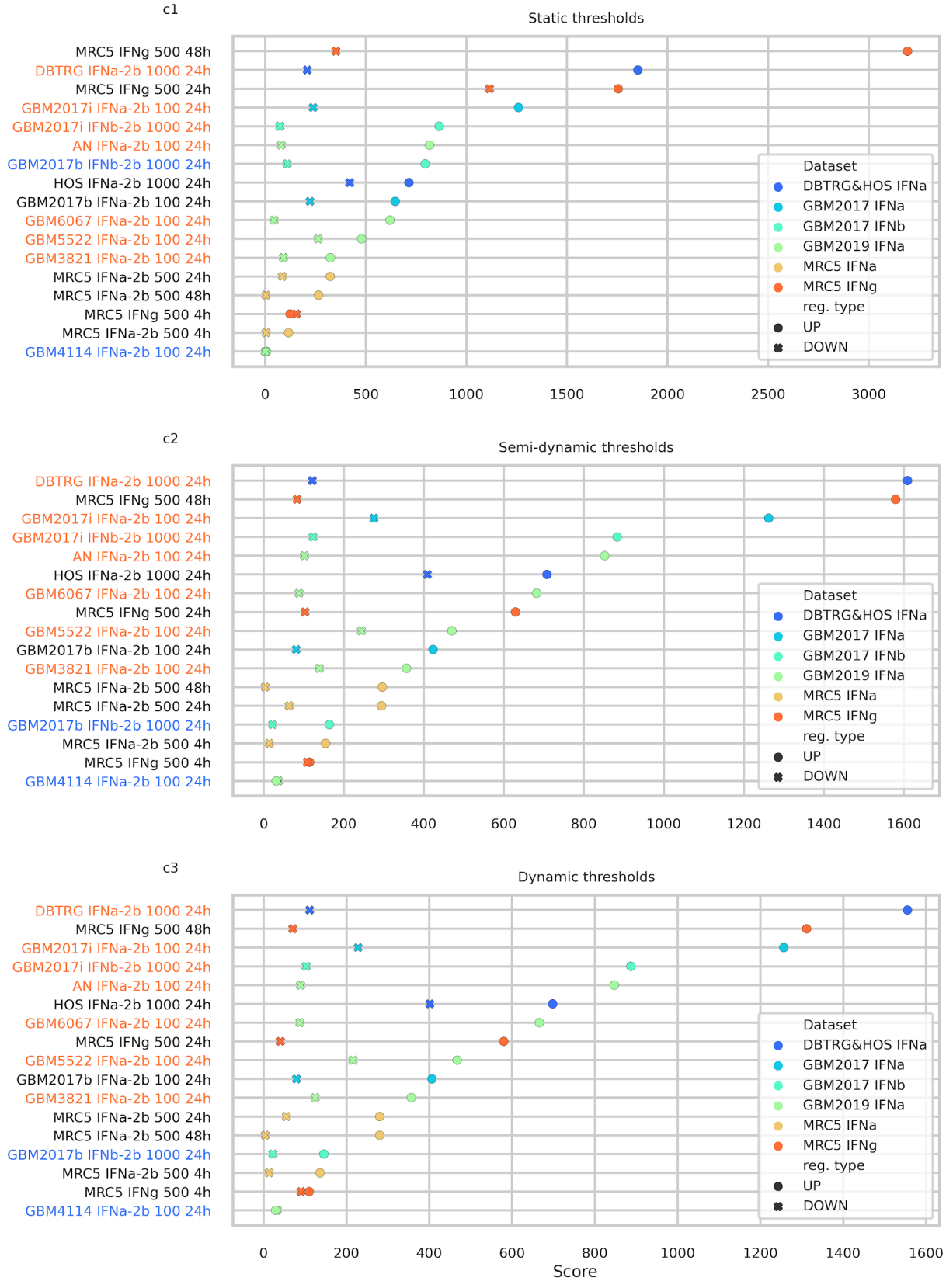
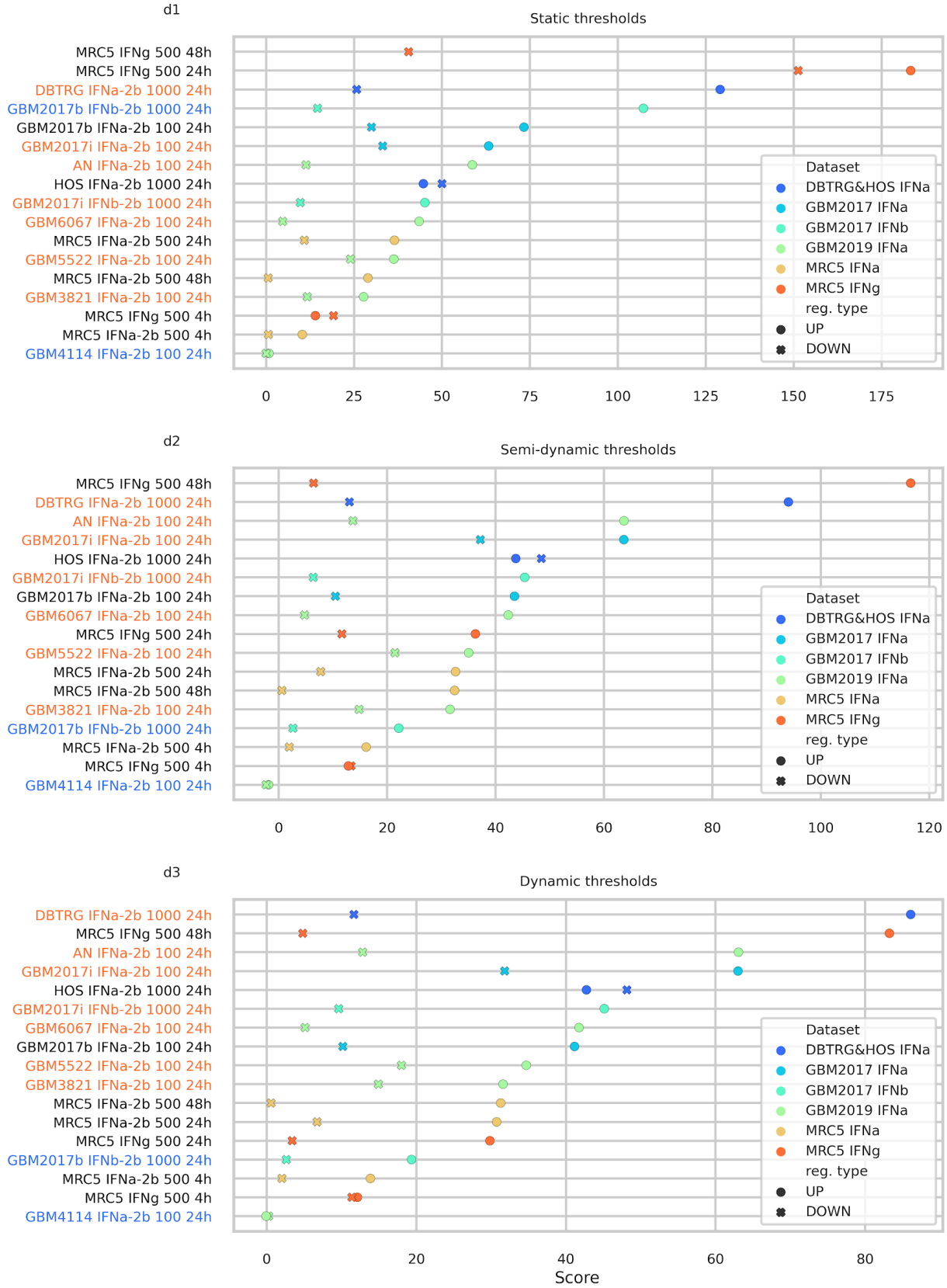
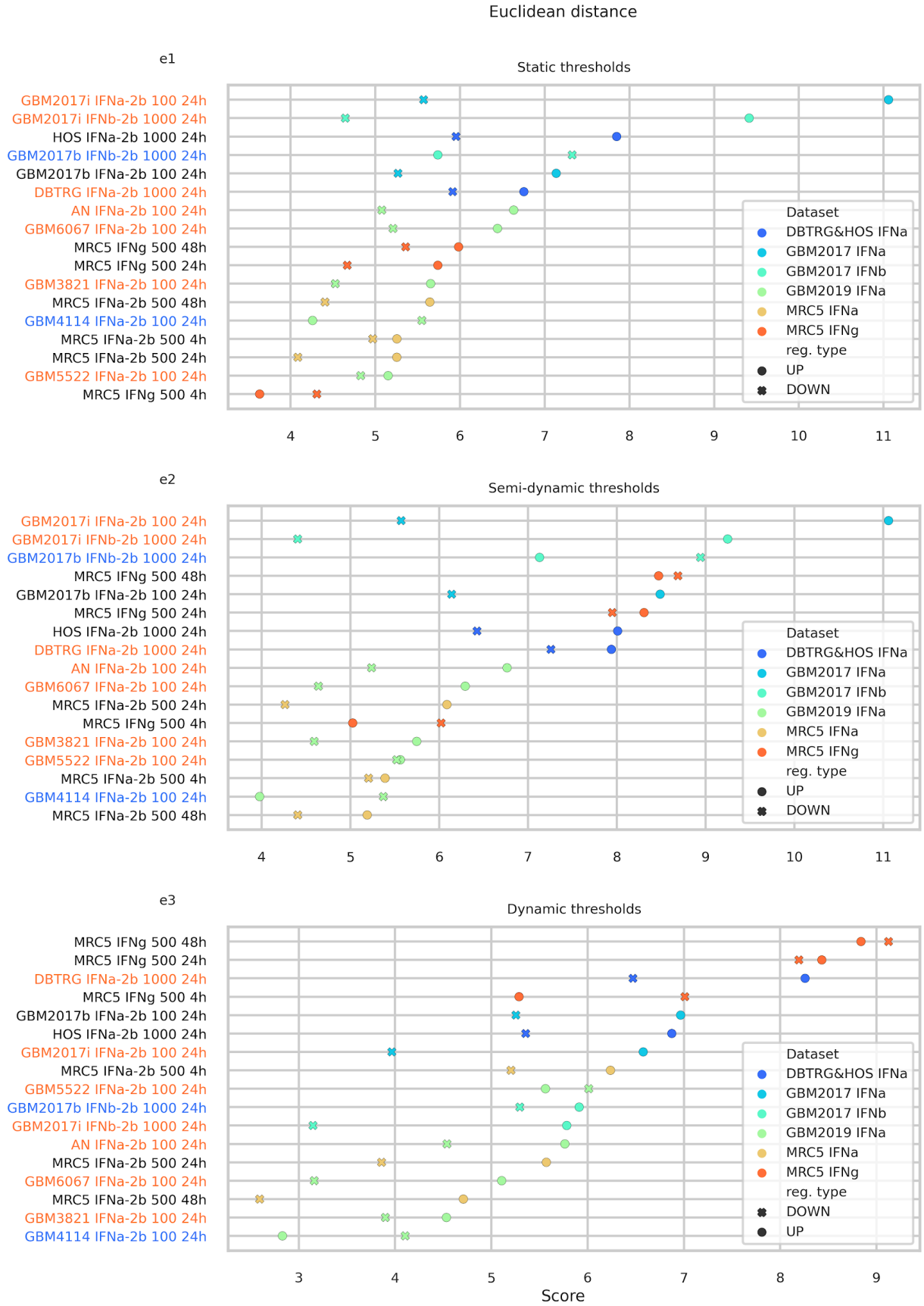
MRC5 IFNa-2b 500 48h
GBM6067 IFNa-2b 100 24h
MRC5 IFNg 500 4h
GBM2017b IFNb-2b 1000 24h
MRC5 IFNa-2b 500 4h
MRC5 IFNa-2b 500 24h
GBM2017b IFNa-2b 100 24h
GBM4114 IFNa-2b 100 24h
Dataset
DBTRG&HOS IFNa
GBM2017 IFNa
GBM2017 IFNb
GBM2019 IFNa
MRC5 IFNa
MRC5 IFNg
reg. type
UP
DOWN
1
2
3
4
5
Score

$$\pi_1 = \sum_{i=1}^{n} | \, log_2 FC_i \cdot log_{10} FDR_i \, |$$



$$\pi_2 = log_{10}(\prod_{i=1}^{n} \mid log_2FC_i \cdot log_{10}FDR_i \mid)$$

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MRC5 IFNg 500 24h
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MRC5 IFNg 500 4h
Dataset
DBTRG&HOS IFNa
GBM2017 IFNa
GBM2017 IFNb
GBM2019 IFNa
MRC5 IFNa
MRC5 IFNg
reg. type
UP
DOWN
4
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e2
Semi-dynamic thresholds
GBM2017i IFNa-2b 100 24h
GBM2017i IFNb-2b 1000 24h
GBM2017b IFNb-2b 1000 24h
MRC5 IFNg 500 48h
GBM2017b IFNa-2b 100 24h
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AN IFNa-2b 100 24h
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MRC5 IFNa-2b 500 24h
MRC5 IFNg 500 4h
GBM3821 IFNa-2b 100 24h
GBM5522 IFNa-2b 100 24h
MRC5 IFNa-2b 500 4h
GBM4114 IFNa-2b 100 24h
MRC5 IFNa-2b 500 48h
Dataset
DBTRG&HOS IFNa
GBM2017 IFNa
GBM2017 IFNb
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reg. type
UP
DOWN
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10
11
e3
Dynamic thresholds
MRC5 IFNg 500 48h
MRC5 IFNg 500 24h
DBTRG IFNa-2b 1000 24h
MRC5 IFNg 500 4h
GBM2017b IFNa-2b 100 24h
HOS IFNa-2b 1000 24h
GBM2017i IFNa-2b 100 24h
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AN IFNa-2b 100 24h
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GBM4114 IFNa-2b 100 24h
Dataset
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UP
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6
7
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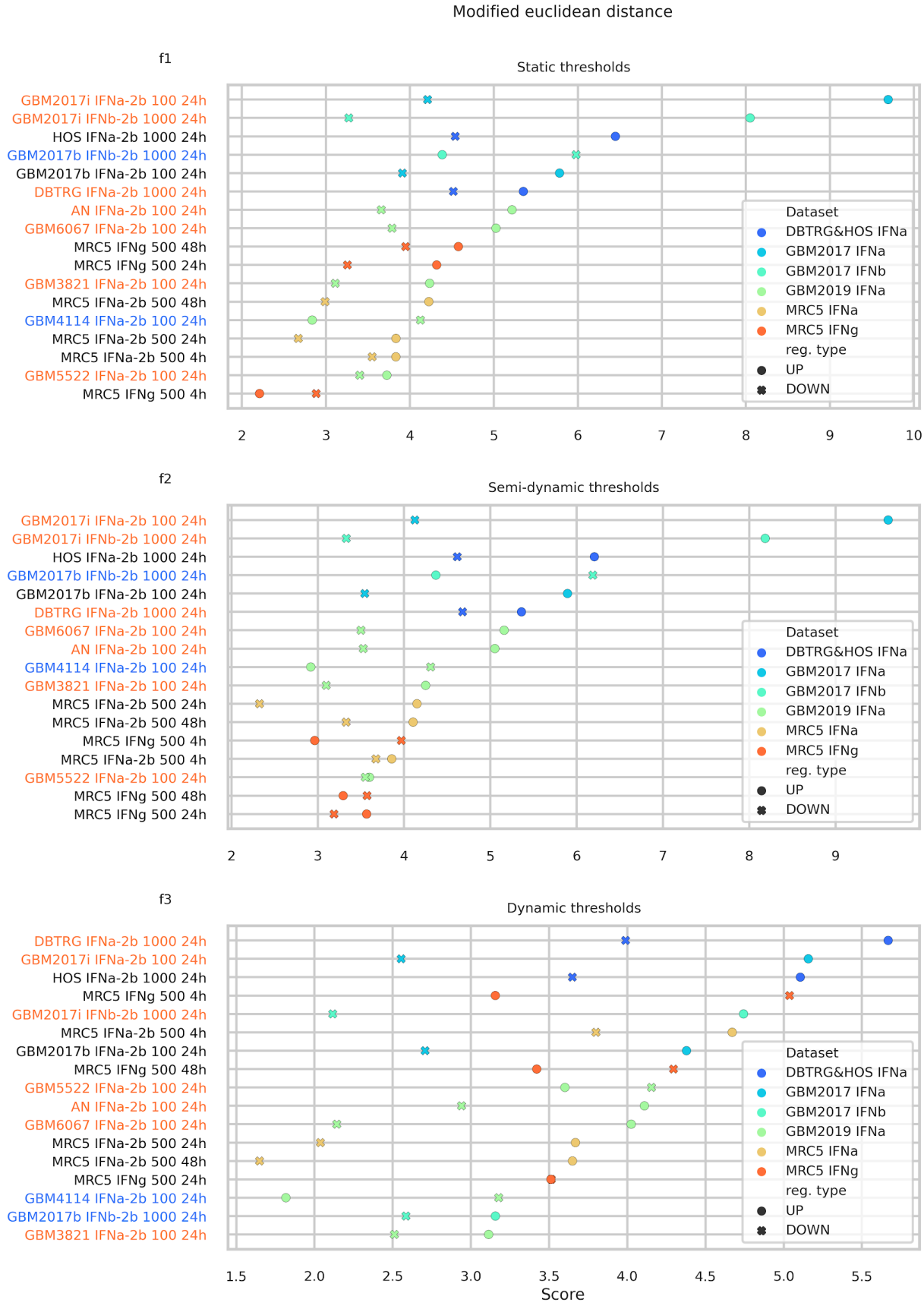
9
Score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HOS IFNa-2b 1000 24h
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AN IFNa-2b 100 24h
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MRC5 IFNg 500 48h
MRC5 IFNg 500 24h
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MRC5 IFNg 500 4h
Dataset
DBTRG&HOS IFNa
GBM2017 IFNa
GBM2017 IFNb
GBM2019 IFNa
MRC5 IFNa
MRC5 IFNg
reg. type
UP
DOWN
2 3 4 5 6 7 8 9 10
f2
Semi-dynamic thresholds
GBM2017i IFNa-2b 100 24h
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HOS IFNa-2b 1000 24h
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GBM5522 IFNa-2b 100 24h
MRC5 IFNg 500 48h
MRC5 IFNg 500 24h
Dataset
DBTRG&HOS IFNa
GBM2017 IFNa
GBM2017 IFNb
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MRC5 IFNa
MRC5 IFNg
reg. type
UP
DOWN
2 3 4 5 6 7 8 9
f3
Dynamic thresholds
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GBM2017i IFNa-2b 100 24h
HOS IFNa-2b 1000 24h
MRC5 IFNg 500 4h
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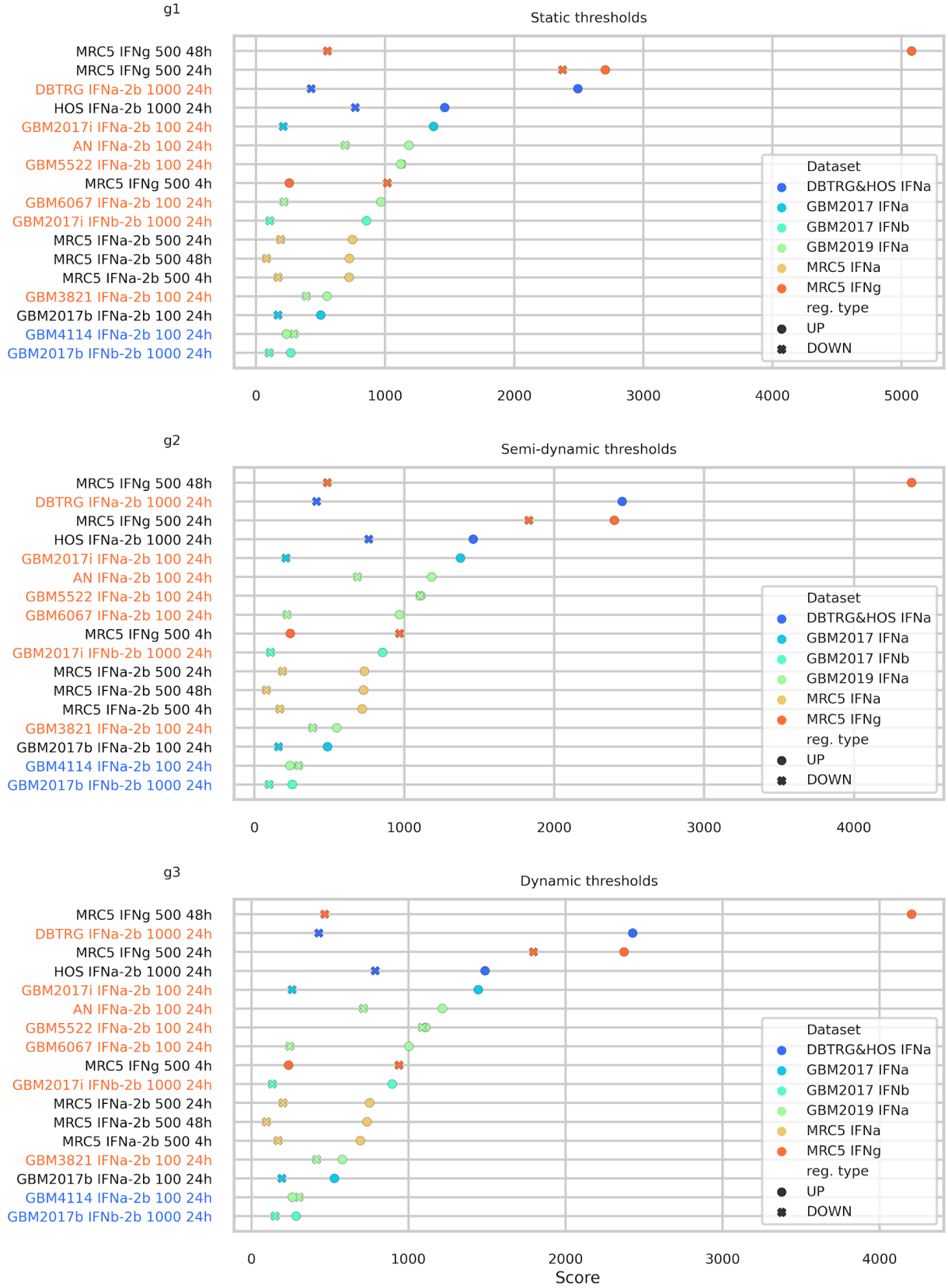
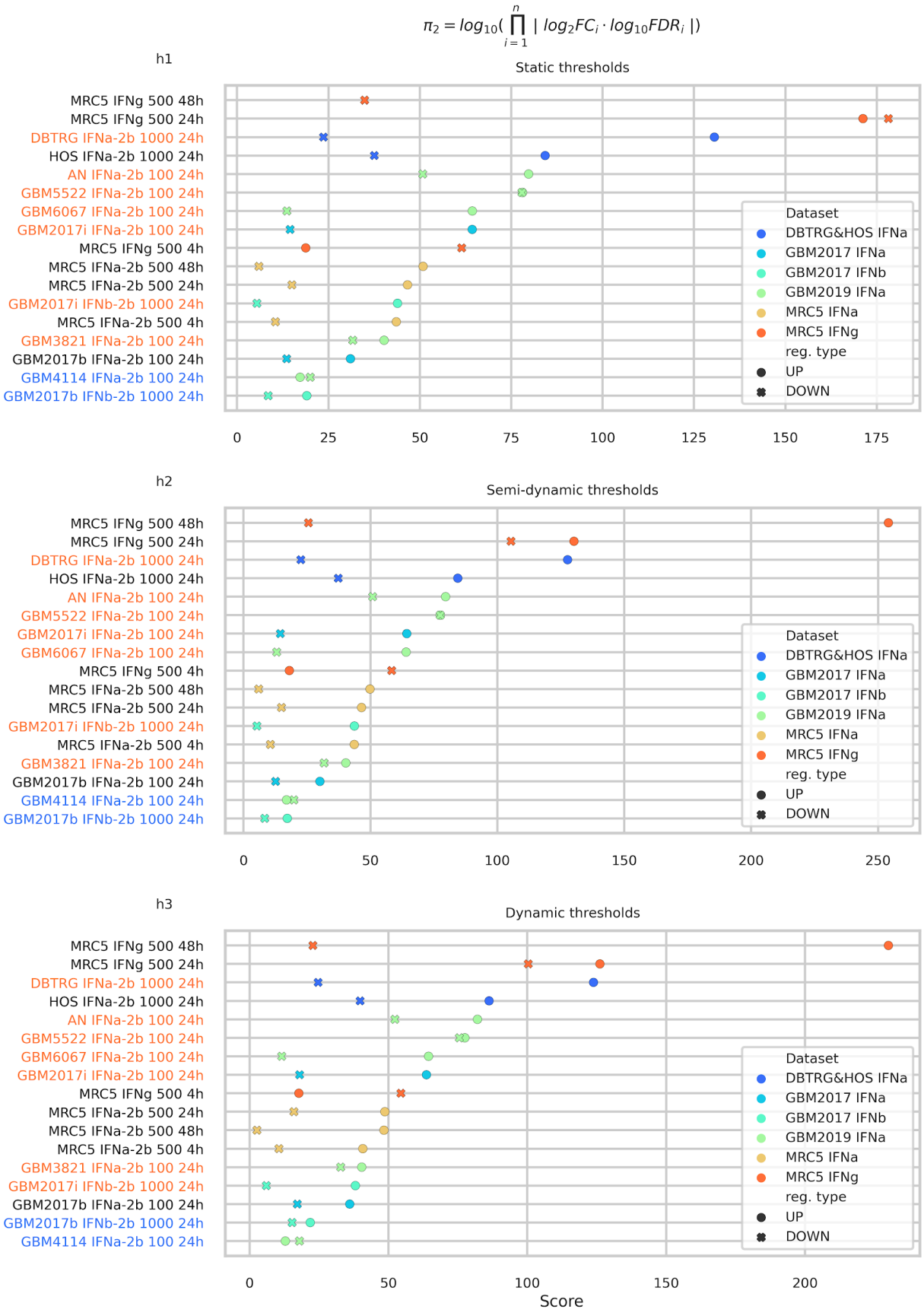
Dataset
DBTRG&HOS IFNa
GBM2017 IFNa
GBM2017 IFNb
GBM2019 IFNa
MRC5 IFNa
MRC5 IFNg
reg. type
UP
DOWN
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
Score

$$\pi_1 = \sum_{i=1}^{n} | \, log_2FC_i \cdot log_{10}FDR_i \, |$$




$\pi_2 = log_{10}(\prod_{i=1}^{n} | log_2FC_i \cdot log_{10}FDR_i |)$
h1
Static thresholds
MRC5 IFNg 500 48h
MRC5 IFNg 500 24h
DBTRG IFNa-2b 1000 24h
HOS IFNa-2b 1000 24h
AN IFNa-2b 100 24h
GBM5522 IFNa-2b 100 24h
GBM6067 IFNa-2b 100 24h
GBM2017i IFNa-2b 100 24h
MRC5 IFNg 500 4h
MRC5 IFNa-2b 500 48h
MRC5 IFNa-2b 500 24h
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75
100
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175
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UP
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MRC5 IFNa-2b 500 48h
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GBM2017b IFNa-2b 100 24h
GBM2017b IFNb-2b 1000 24h
GBM4114 IFNa-2b 100 24h
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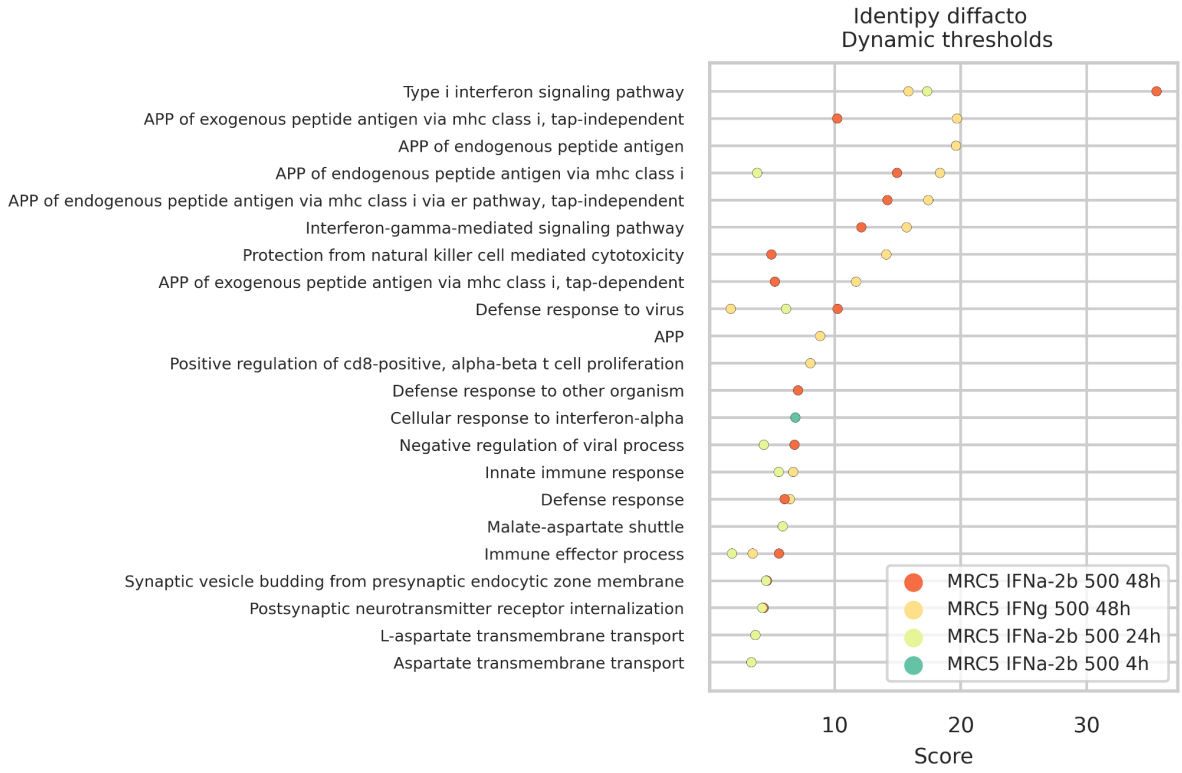
Score
Dataset
DBTRG&HOS IFNa
GBM2017 IFNa
GBM2017 IFNb
GBM2019 IFNa
MRC5 IFNa
MRC5 IFNg
reg. type
UP
DOWN

Figure S1. Ranking cellular response to type I and type II interferon treatment using different score equations, missing value imputation strategies (imputation with k-nearest neighbors machine learning or imputation with minimal NSAF detected in LC-MS/MS replicate) and thresholds for DRF selection of (static, semi dynamic or dynamic). Negative controls (blue text on Y axis) clusterized below the positive ones (red text on Y axis) differentiate the best selectivity for response scoring, imputation and DRF selection strategies. DRF selection: static thresholds - DRF selection with *fdr* < 0.05, $|\log_2 FC| > 0.585$; semi dynamic - DRF selection with $|\log_2 FC| > 0.585$ and $X_1 = Q_1 - 1.5(Q_3 - Q_1)$; dynamic thresholds stand for *fdr* and FC determined as $X_1 = Q_1 - 1.5(Q_3 - Q_1)$, $X_2 = Q_1 + 1.5(Q_3 - Q_1)$ based on $-\log_{10} fdr$ and $\log_2 FC$ density plots. Designation of subfigures in **Fig. S1** corresponds to **Table 2** in the main manuscript, summarizing the clusterization of positive (Pos) and negative (Neg) controls for different data processing strategies. "True" stands if all positive controls are ranked with higher scores than the negative ones.

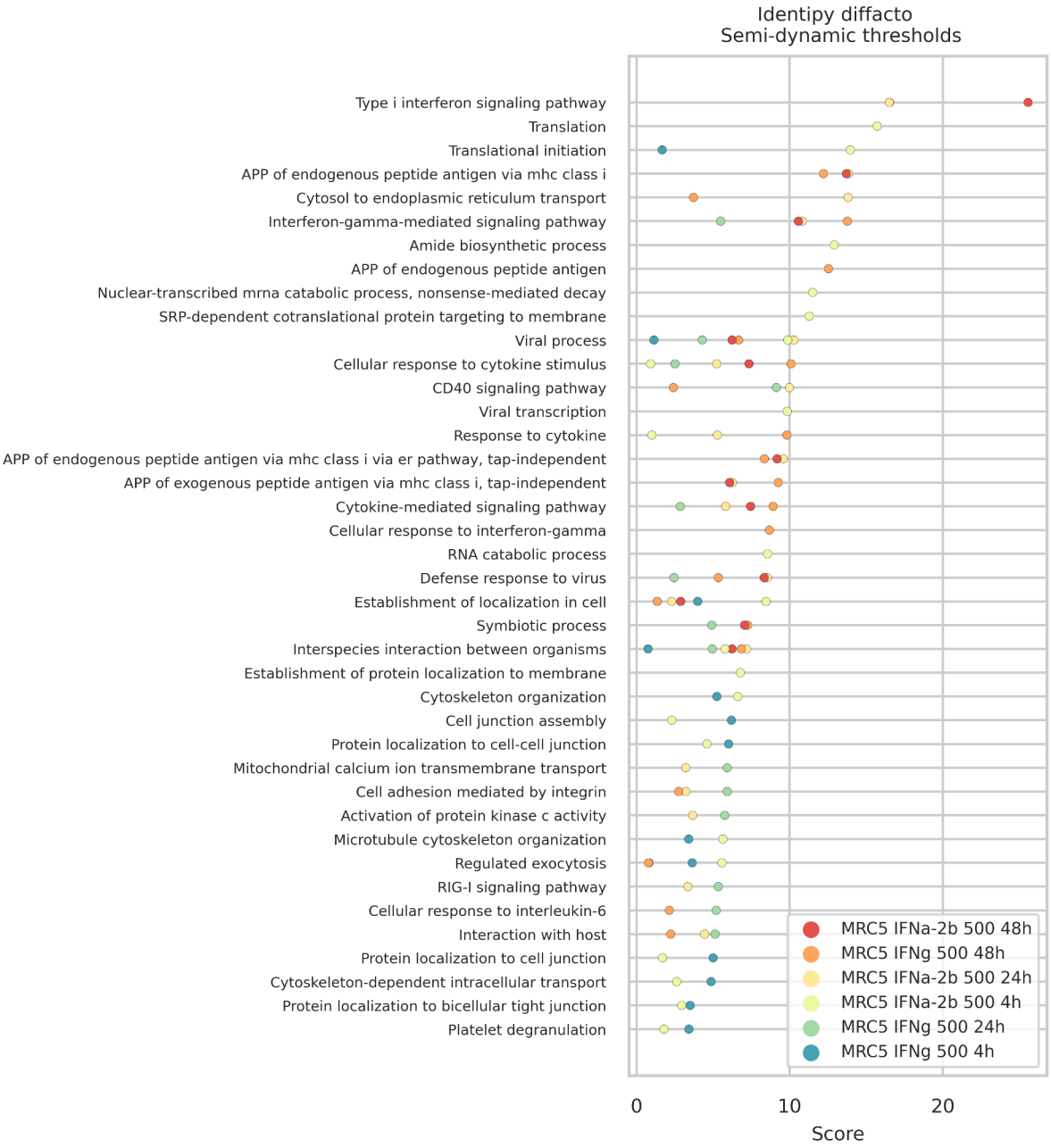
Ref. Fig.S1	Imputation	Equation	DRF selection	Pos above Neg?
a1	Minimal NSAF	E	static	False
a2			semi dyn	False
a3			dyn	False
b1		E_m	static	False
b2			semi dyn	True
b3			dyn	True
c1		π_1	static	False
c2			semi dyn	True
c3			dyn	True
d1		π_2	static	False
d2			semi dyn	True
d3			dyn	True
e1	kNN	E	static	False
e2			semi dyn	False
e3			dyn	False
f1		E_m	static	False
f2			semi dyn	False
f3			dyn	False
g1		π_1	static	True
g2			semi dyn	True
g3			dyn	True
h1		π_2	static	True
h2			semi dyn	True
h3			dyn	True

Figure S2. Top enriched biological processes ranked by GO score. IdentiPy [DOI: 10.1021/acs.jproteome.7b00640] and Scavager [DOI: 10.1002/pmic.201800280] were used for peptide spectrum matching and postsearch validation. LFQ was performed using Diffacto [DOI: 10.1074/mcp.O117.067728]. Differentially regulated proteins were selected using (a) dynamic, (b) semi dynamic or (c) static, *fdr* < 0.05 and $|\log_2 FC| > 0.585$, thresholds. STRING [doi: 10.1093/nar/gky1131] was used for GO analysis. $GO_{score} = E \cdot abs(log_{10} fdr)$, where E and *fdr* are the enrichment and Benjamini-Hochberg corrected *p*-values, respectively, for the enriched GO term reported by STRING. "APP" stands for antigen processing and presentation.

(a)



(b)


Identipy diffacto
Semi-dynamic thresholds
Type i interferon signaling pathway
Translation
Translational initiation
APP of endogenous peptide antigen via mhc class i
Cytosol to endoplasmic reticulum transport
Interferon-gamma-mediated signaling pathway
Amide biosynthetic process
APP of endogenous peptide antigen
Nuclear-transcribed mrna catabolic process, nonsense-mediated decay
SRP-dependent cotranslational protein targeting to membrane
Viral process
Cellular response to cytokine stimulus
CD40 signaling pathway
Viral transcription
Response to cytokine
APP of endogenous peptide antigen via mhc class i via er pathway, tap-independent
APP of exogenous peptide antigen via mhc class i, tap-independent
Cytokine-mediated signaling pathway
Cellular response to interferon-gamma
RNA catabolic process
Defense response to virus
Establishment of localization in cell
Symbiotic process
Interspecies interaction between organisms
Establishment of protein localization to membrane
Cytoskeleton organization
Cell junction assembly
Protein localization to cell-cell junction
Mitochondrial calcium ion transmembrane transport
Cell adhesion mediated by integrin
Activation of protein kinase c activity
Microtubule cytoskeleton organization
Regulated exocytosis
RIG-I signaling pathway
Cellular response to interleukin-6
Interaction with host
Protein localization to cell junction
Cytoskeleton-dependent intracellular transport
Protein localization to bicellular tight junction
Platelet degranulation
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MRC5 IFNg 500 48h
MRC5 IFNa-2b 500 24h
MRC5 IFNa-2b 500 4h
MRC5 IFNg 500 24h
MRC5 IFNg 500 4h
0
10
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Score

(c)


Identipy diffacto
Static thresholds
Translational initiation
Translation
Viral process
SRP-dependent cotranslational protein targeting to membrane
Viral gene expression
Symbiotic process
Peptide metabolic process
Nuclear-transcribed mrna catabolic process, nonsense-mediated decay
Amide biosynthetic process
Establishment of protein localization to endoplasmic reticulum
Establishment of localization in cell
Cellular localization
Cellular amide metabolic process
Peptide biosynthetic process
Intracellular protein transport
APP of exogenous antigen
APP
Neutrophil degranulation
Leukocyte degranulation
Myeloid leukocyte mediated immunity
Myeloid cell activation involved in immune response
Regulated exocytosis
Establishment of protein localization
Exocytosis
Wnt signaling pathway, planar cell polarity pathway
Generation of precursor metabolites and energy
Cellular protein localization
Cellular respiration
ATP metabolic process
APP of endogenous peptide antigen via mhc class i
Respiratory electron transport chain
Oxidative phosphorylation
Type i interferon signaling pathway
Electron transport chain
Mitochondrial atp synthesis coupled electron transport
APP of endogenous peptide antigen
Aerobic respiration
Cytosol to endoplasmic reticulum transport
Purine ribonucleotide transport
Interaction with host
Interferon-gamma-mediated signaling pathway
Entry into host
MRC5 IFNa-2b 500 4h
MRC5 IFNg 500 48h
MRC5 IFNg 500 4h
MRC5 IFNg 500 24h
MRC5 IFNa-2b 500 24h
MRC5 IFNa-2b 500 48h
0
20
40
Score

Figure S3. Heatmap for the proteins involved in biological processes correlated with topotecan concentrations, Pearson coeff. = 0.9 (GO terms shown in Fig.5 of the manuscript).