Supplemental Materials

Molecular Biology of the Cell

Hood et al.

Supplementary Figure 1. *Oncogenic Ras is active in the absence of growth factor stimulation.* (A) Ras activity in isogenic SW48 cells measured using a Raf RBD assay. GDP represents 100% inactive Ras and GTPγS indicates 100% active Ras. (B) Quantification of Ras activity (n=3) indicates that all G12V mutated isoforms are significantly activated versus wild type control cells. (C) Mutated Ras isoforms display impaired responsiveness to EGF stimulation. Western blot data are representative of n=3 experiments. Graphs depict mean values ±SEM; paired t-test, * $p<0.05$, ** $p<0.01$.

Supplementary Figure 2. *Profiling Ras signaling in available SW48 cell G12V Ras clones.* Evidence for some clonalityis seen with $KRAS^{G12V}$ cells. In contrast, the Ras signaling outputs from $NRAS^{G12V}$ clones are largely homogenous. Cells were grown under standard cell culture conditions in the presence of 10% FBS. Western blotting data representative of n=4-7 biological replicates.Graphs of quantified Western blots depict mean values ±SEM; paired t-test, * $p<0.05$.

Supplementary Figure 3. *Luminex measurement of Ras network outputs in the panel of isogenic SW48 cells.* (A) Luminex analysis of Ras signaling pathway nodes reveals a lack of network activation versus the Parental control in serum-starved isogenic SW48 cells.Data points from technical and biological replicates are shown (circles), bars represent mean ± SD from n=2 experiments each comprising n=3-8 technical replicates.(B) An alternative presentation of data from Figure 1D with data grouped by cell line. Data points indicating n=2 biological

replicates.p-values correspond to Tukey's test (for A versus Parental, for B versus untreated) for those cases where multiple testing corrected one-way-ANOVA was significant (FDR≤0.05); * p<0.05, **, p<0.01, *** p<0.001.

Supplementary Figure 4. *An alternative presentation of a subset of data from Figure 2B grouped by cell line.*

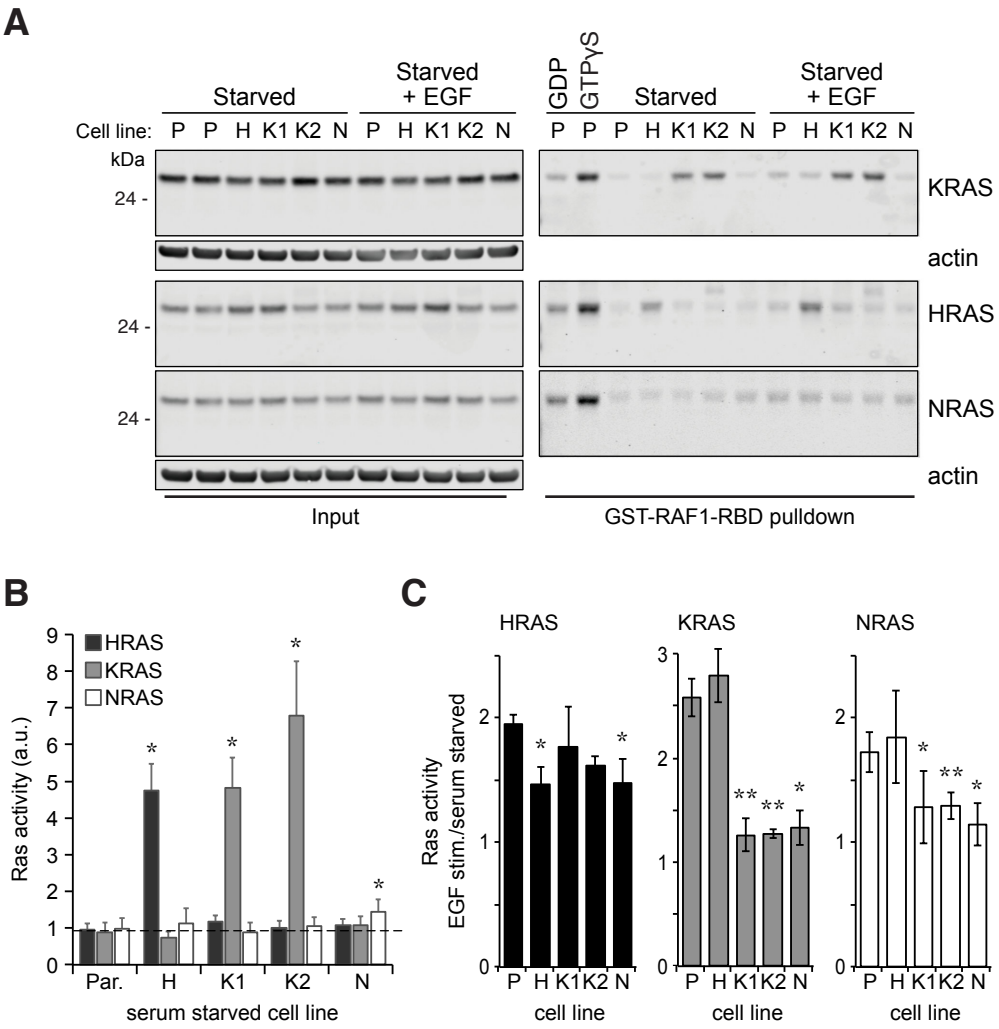
Supplementary Figure 5. *Parameter heatmap where Ras mutations are modeled as perturbations.* All mutations exert a negative influence on signaling. In the presence (all data) and absence of growth factors (no GF). Basal signaling versus wild type Ras cells trends downwards for both PI3K and RAF pathways for all isoforms and shows no clear differential isoform-specific effect on downstream outputs(for modeling details refer to Material and Methods and Supplemental File 2).

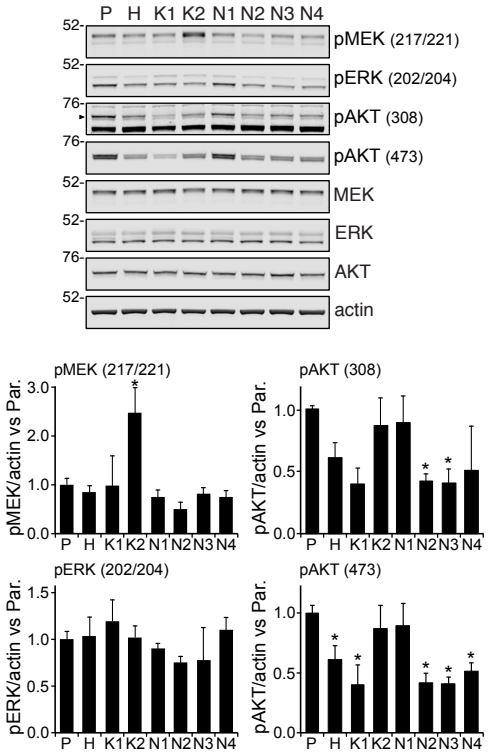
Supplementary Figure 6. *ERK-dependent negative feedback to CRAF is independent of the presence of oncogenic Ras mutants.* Blots are from a single experiment and representative of n=3 biological replicates. Graphs depict mean values ±SEM; n=3; paired t-test versus Parental cells or indicated pair-wise comparisons, * p<0.05, ** p<0.01, *** p<0.001. Cells starved for 24 hours, ± 15ng/ml EGF stimulation for 20 minutes, ±5μM AZD6244 MEK inhibitor, 60 minutes pre-treatment and co-incident incubation during the EGF stimulation.

Supplementary File 1. *SW48 Ras isoforms: Normalization.* Code for normalizing plate to plate variability and pre- and post-normalization data.

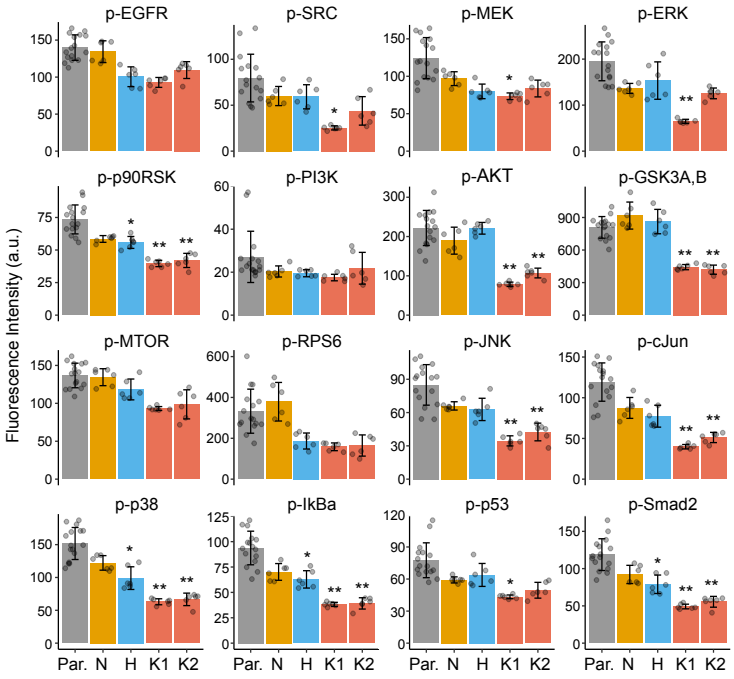
Supplementary File 2. *Modelling workflow.*Code and workthrough of the modeling steps for network structure determination.

Supplementary File 3. *Ras activity screen.* Colorectal cell line panel signaling readout analysis html containing source data.

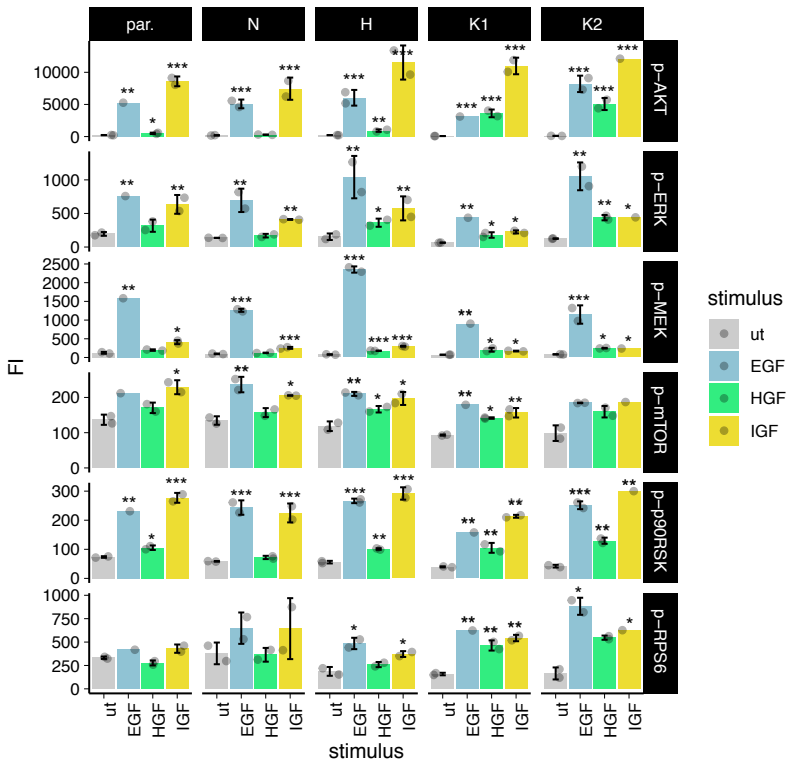

A
Starved
Starved + EGF
GDP
GTPγS
Starved
Starved + EGF
Cell line: P P H K1 K2 N P H K1 K2 N P P P H K1 K2 N P H K1 K2 N
kDa
24 -
KRAS
actin
HRAS
NRAS
Input
GST-RAF1-RBD pulldown
B
HRAS
KRAS
NRAS
Ras activity (a.u.)
Par. H K1 K2 N
serum starved cell line
C
HRAS
KRAS
NRAS
Ras activity
EGF stim./serum starved
P H K1 K2 N
cell line

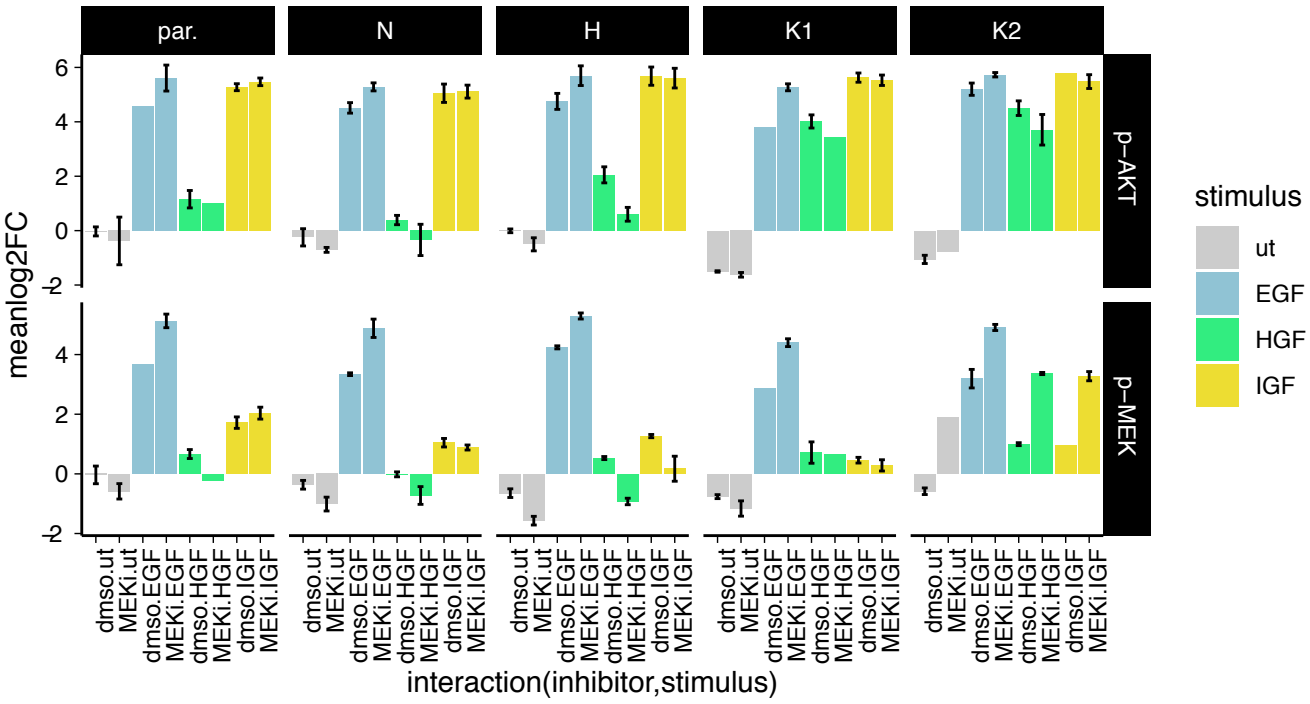

P H K1 K2 N1 N2 N3 N4
52-
pMEK (217/221)
52-
pERK (202/204)
76-
pAKT (308)
76-
pAKT (473)
52-
MEK
52-
ERK
76-
AKT
52-
actin
pMEK (217/221)
pMEK/actin vs Par.
3.0
2.0
1.0
0.0
pAKT (308)
pAKT/actin vs Par.
1.0
0.5
0.0
pERK (202/204)
pERK/actin vs Par.
1.5
1.0
0.5
0.0
pAKT (473)
pAKT/actin vs Par.
1.0
0.5
0.0
P H K1 K2 N1 N2 N3 N4

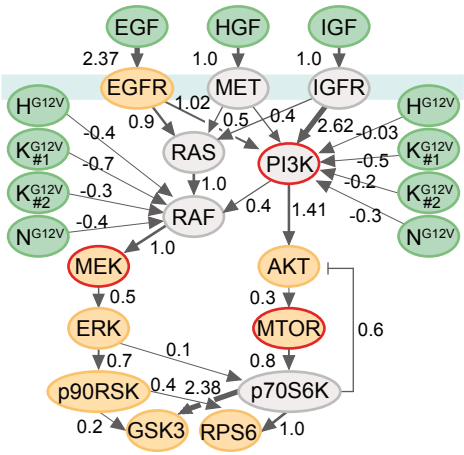
A

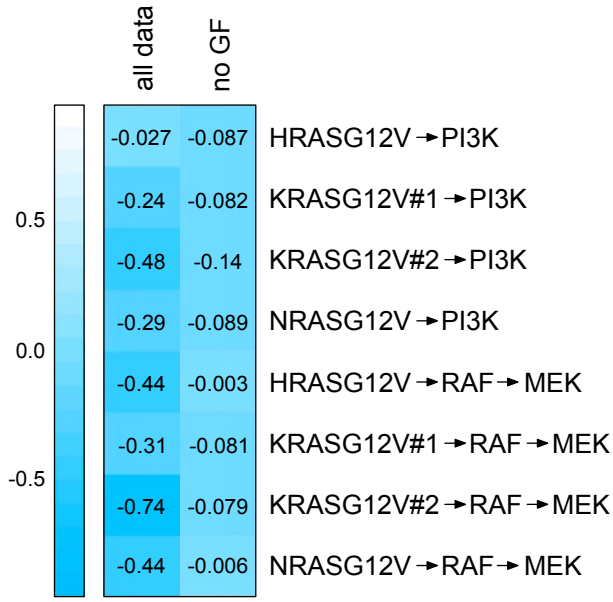

p-EGFR
p-SRC
p-MEK
p-ERK
p-p90RSK
p-PI3K
p-AKT
p-GSK3A,B
p-MTOR
p-RPS6
p-JNK
p-cJun
p-p38
p-IkBa
p-p53
p-Smad2
Fluorescence Intensity (a.u.)
Par. N H K1 K2

B


par.
N
H
K1
K2
p-AKT
p-ERK
p-MEK
p-mTOR
p-p90RSK
p-RPS6
FI
ut EGF HGF IGF
stimulus
stimulus
ut
EGF
HGF
IGF


par.
N
H
K1
K2
p−AKT
p−MEK
meanlog2FC
6
4
2
0
−2
dmso.ut
MEKi.ut
dmso.EGF
MEKi.EGF
dmso.HGF
MEKi.HGF
dmso.IGF
MEKi.IGF
interaction(inhibitor,stimulus)
stimulus
ut
EGF
HGF
IGF


EGF
HGF
IGF
2.37
1.0
1.0
EGFR
MET
IGFR
H G12V
K G12V #1
K G12V #2
N G12V
1.02
0.9
0.5
0.4
2.62
0.03
-0.4
-0.7
-0.3
-0.4
RAS
PI3K
RAF
1.0
0.4
1.41
-0.5
-0.2
-0.3
1.0
MEK
AKT
0.5
0.3
ERK
MTOR
0.6
0.1
0.7
0.8
p90RSK
p70S6K
0.4
2.38
0.2
GSK3
RPS6
1.0


all data
no GF
0.5
0.0
-0.5
-0.027
-0.087
HRASG12V → PI3K
-0.24
-0.082
KRASG12V#1 → PI3K
-0.48
-0.14
KRASG12V#2 → PI3K
-0.29
-0.089
NRASG12V → PI3K
-0.44
-0.003
HRASG12V → RAF → MEK
-0.31
-0.081
KRASG12V#1 → RAF → MEK
-0.74
-0.079
KRASG12V#2 → RAF → MEK
-0.44
-0.006
NRASG12V → RAF → MEK

ERK-RAF negative feedback phosphorylation