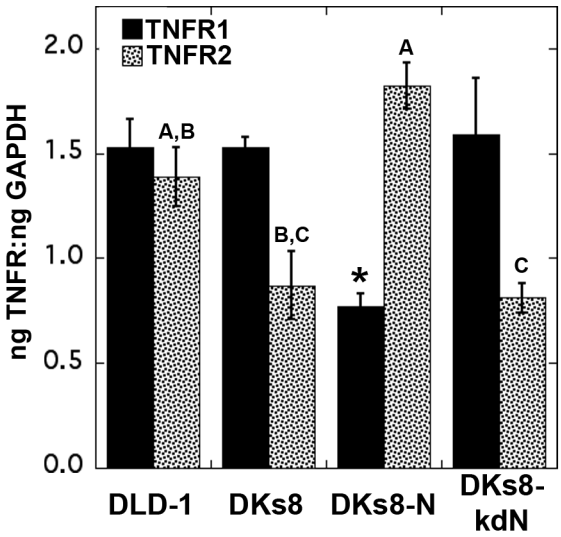


**Figure S1.** TNFR1 and TNFR2 expression relative to GAPDH. mRNA was isolated from IFN-γ-sensitized cells using RNeasy Mini kits (Qiagen, Valencia, CA) and quantified on a NanoDrop (Thermo Scientific, Waltham, MA). 1µg of RNA was transcribed to cDNA using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA). 25ng of cDNA was analyzed by RT-PCR using QuantiTect SYBR Green and Primers (Qiagen) on a 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA). For TNFR1, DKs8-N cells have significantly decreased levels compared to the other three cell lines (indicated by *, Tukey-HSD, $p<0.05$). TNFR2 levels also vary between the cell lines (cell lines without a common letter are significantly different, Tukey-HSD, $p<0.05$).

Although the cell lines show different expression patterns of TNFR1 (which contains a death domain) and TNFR2 (a soluble form that may antagonize TNFR1 action), these patterns do not correlate precisely to the extent of apoptosis (1). For example, DKs8 (a cell line with low apoptosis) and DKs8-kdN (a cell line with high apoptosis) have similar expression profiles. These observations motivate the examination of the impact of RAS mutants on downstream kinases that interpret the input from the TNF receptors.

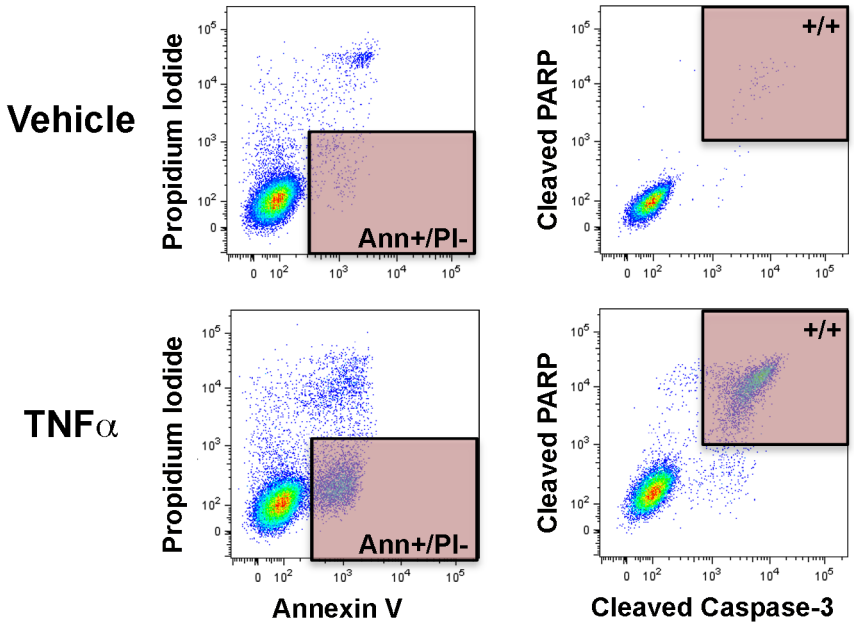


**Figure S2.** Example gatings for the two apoptotic assays for DLD-1 cells at 12 hours. The Annexin+/propidium iodide- population is an early event in apoptosis, while cleaved caspase-3+/cleaved PARP+ cells are a later event (2). Alexa-350 Annexin V (1:20) and propidium iodide (1 µg/mL, Invitrogen) were used to stain half of the collected cells. The remaining cells were fixed with 4% paraformaldehyde, permeabilized and stained using anti-cleaved caspase-3 (1:500) and anti-cleaved PARP (1:250, BD Pharmingen, Franklin Lakes, NJ), followed by Alexa 488-donkey-anti-rabbit IgG and Alexa 647-donkey-anti-mouse IgG (both at 1:250, Invitrogen).

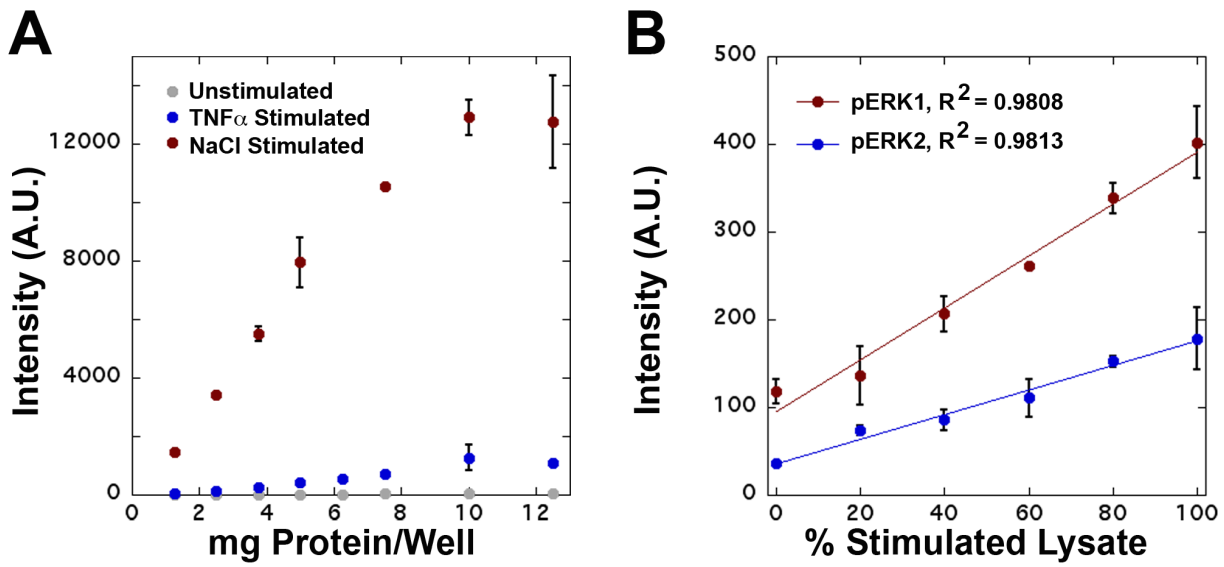


**Figure S3.** Luminex assays for phosphorylated proteins are linear, allowing quantitative comparisons. *A,* Increasing amounts of lysate show a linear response for pHSP27 levels across three different stimuli. *B,* When lysates from strong and weak stimuli are mixed at a set concentration, the level of phosphorylated protein detected is linear (assayed at 5 µg per well). For the collected data set,1.8µg (pAKT) or 5µg (all others) of clarified lysate was assayed for each sample in duplicate.

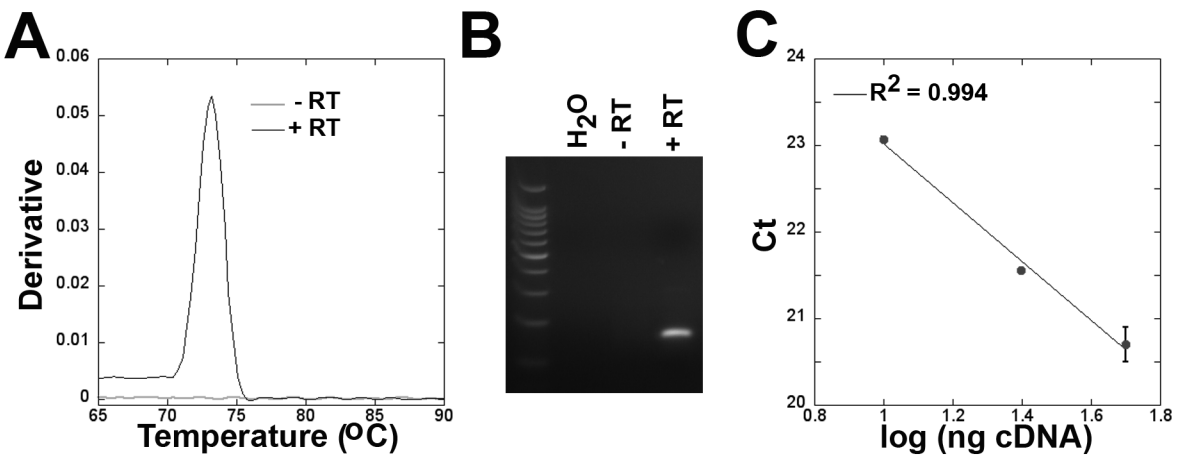


**Figure S4.** mRNA was isolated using RNeasy Mini kits (Qiagen) and quantified on a NanoDrop (Thermo Scientific). 1µg of RNA was transcribed to cDNA using the SuperScript III First-Strand Synthesis System (Invitrogen). 25ng of cDNA was analyzed by RT-PCR using QuantiTect SYBR Green and Primers (Qiagen) on a 7500 Fast Real-Time PCR system (Applied Biosystems). Each DUSP primer set was validated *A,* for clean melting curves*, B,* production of a band of the correct length by gel electrophoresis*,* and *C,* linearity for increasing levels of cDNA. Data shown is for DUSP6.

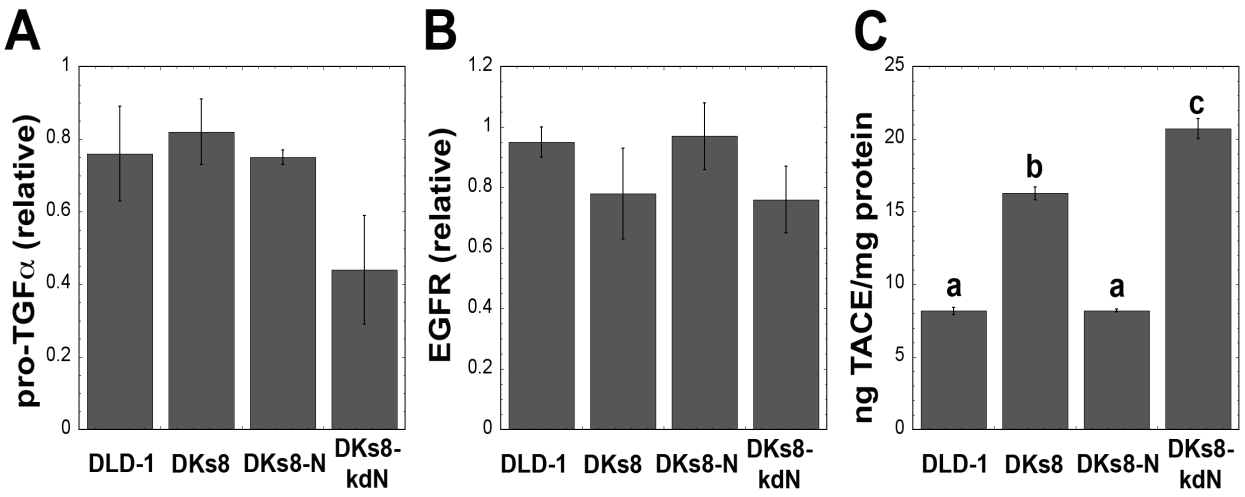


**Figure S5.** RAS-variant cell lines had differences in levels of TACE, but not pro-TGFα or EGFR. *A,B,* Whole cell lysates were examined by quantitative Western Blot for pro-TGFα and EGFR (Cell Signaling Technology, Danvers, MA, #3715, #2232). 15μg of total protein was loaded per lane. Background-subtracted signal was normalized to concurrently measured GAPDH (Cell Signaling Technology #2118). Blots were probed with IR-dye labeled secondary antibodies and detected using the LI-COR Odyssey (LI-COR, Lincoln, NE). Normalized levels were not significantly different, $p>0.05$. *C,* Cell lysates were analyzed for levels of TACE by ELISA (R&D Systems). Levels were normalized to total cellular protein measured by BCA assay (Bio-Rad). Different letters represent significantly different levels by TUKEY-HSD, $p<0.05$.

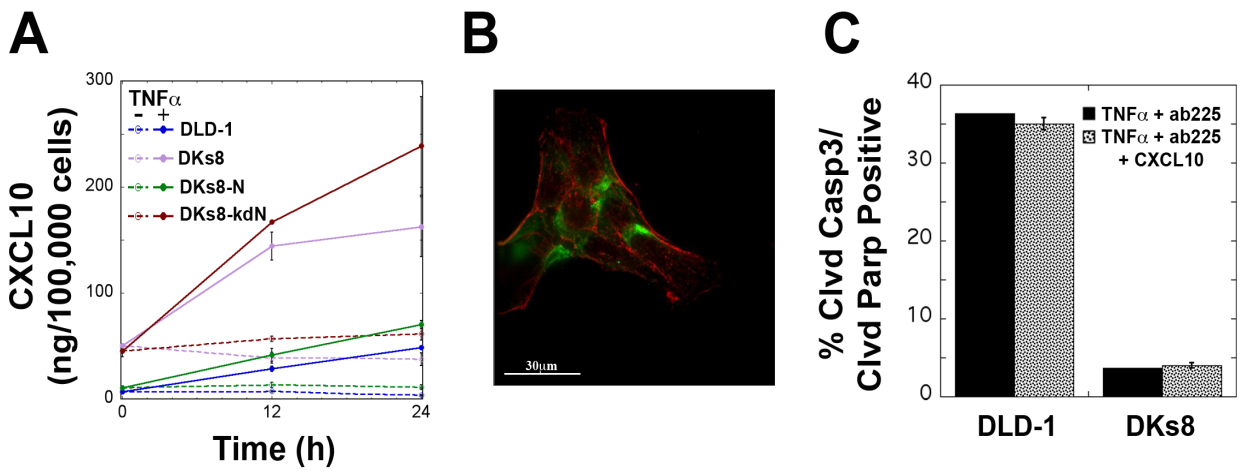


**Figure S6.** RAS variant cells express CXCR3 and produce CXCL10 in response to TNFα, but the potential autocrine loop does not appear to impact apoptosis. *A,* CXCL10 was quantified by Luminex assay and normalized to concurrent cell counts. *B,* CXCR3 was observed in DLD-1 cells by immunofluorescence (described in materials and methods, using 1:5 anti-CXCR3, R&D Systems). Green = CXCR3; Red = phalloidin for actin filaments. *C,* Co-treatment with ab225 decreases CXCL10 levels (data not shown). However, exogenous treatment with CXCL10 to supplement TNFα + ab225 treated cells had no effect on apoptosis at 24 hours. Cells were stained for cleaved caspase-3 and cleaved PARP and analyzed by flow cytometry for double positive (apoptotic) cells.

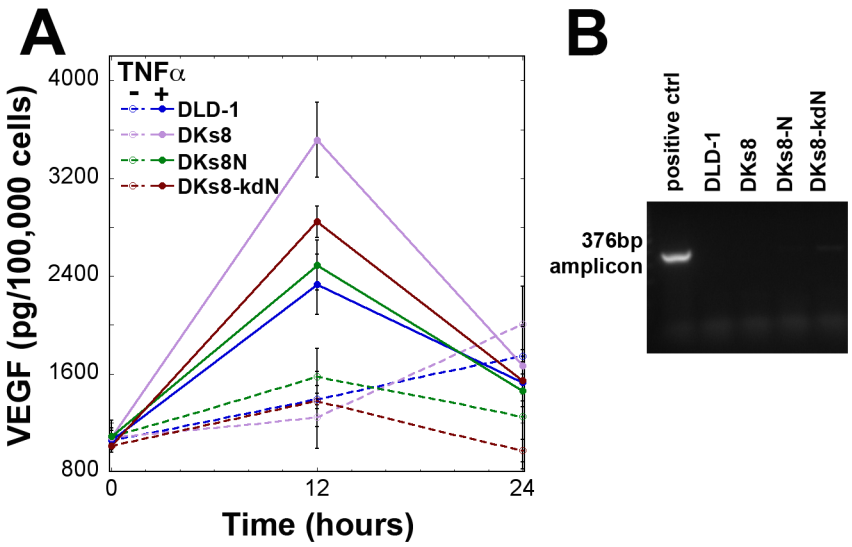


**Figure S7.** RAS variant cells produce VEGF in response to TNFα, but do not express the VEGF-R2. *A,* VEGF was quantified by Luminex assay and normalized to concurrent cell counts. *B,* VEGF-R2 cDNA was not detected following 35 cycles of PCR.

**Supplementary Table S1.** DUSP Screen

| | ΔCt relative to t=0 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | DLD-1 | | | Dks8 | | | Dks8N | | |
| | 30 | 90 | 240 | 30 | 90 | 240 | 30 | 90 | 240 |
| DUSP1 | -0.72 | -0.62 | -0.36 | -1.26 | -1.38 | -0.36 | -0.44 | -0.29 | -0.75 |
| DUSP2 | 0.36 | 0.75 | 0.85 | -0.69 | -1.04 | -0.16 | -0.47 | -0.08 | 0.65 |
| DUSP3 | 0.23 | 0.18 | 0.30 | -0.86 | -1.38 | -1.21 | 0.07 | 0.77 | 0.62 |
| DUSP4 | 0.44 | -0.16 | 0.34 | -0.39 | -1.38 | -1.33 | -0.46 | -0.66 | -0.81 |
| DUSP5 | -1.23 | -1.08 | -0.75 | -1.39 | -3.26 | -2.78 | -0.46 | -1.98 | -2.24 |
| DUSP6 | -0.01 | -0.21 | 0.34 | -0.11 | -0.66 | -0.27 | -0.25 | -0.04 | 0.16 |
| DUSP7 | 0.04 | 0.05 | 0.76 | -0.13 | -0.89 | -0.52 | -0.33 | -0.20 | -0.10 |
| DUSP9 | Levels too low to reliably detect | | | | | | | | |
| DUSP14 | 0.27 | 0.49 | 0.50 | 0.37 | 0.15 | 0.43 | 0.10 | 0.45 | 0.23 |

Expression levels of DUSP genes were screened by RT-PCR for nine phosphatases that recognize ERK as a substrate (3). Each RAS-variant line was plated and treated with IFN$\gamma$ and TNF$\alpha$ as described in the materials and methods. At time 0, 30, 90, and 240 minutes, mRNA was isolated using RNeasy Mini kits (Qiagen) and quantified on a NanoDrop (Thermo Scientific). 1$\mu$g of RNA was transcribed to cDNA using the SuperScript III First-Strand Synthesis System (Invitrogen). 25ng of cDNA was analyzed by RT-PCR using QuantiTect SYBR Green and Primers (Qiagen) on a 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA). Only DUSP5 showed a consistent up-regulation across the three RAS-variants examined.

**Supplementary Table S2.** Top 20 most informative signals in the PLSR models.

| Apoptosis | | | CXCL1 and CXCL8 | | |
|---|---|---|---|---|---|
| VIP[1] | Signal | Time (min) | VIP | Signal | Time (min) |
| 1.49 | Clvd Casp-8 (MW = 41) | 720 | 1.39 | pJNK | 15 |
| 1.44 | pIκBα | 60 | 1.36 | pJNK | 30 |
| 1.44 | Clvd Casp-8 (MW = 41) | 480 | 1.35 | pIκBα | 120 |
| 1.39 | pIκBα | 240 | 1.35 | pHSP27 | 15 |
| 1.39 | pHSP27 | 0 | 1.35 | pIκBα | 90 |
| 1.35 | pERK2 | 720 | 1.35 | pHSP27 | 30 |
| 1.32 | pJNK | 0 | 1.33 | pHSP27 | 60 |
| 1.31 | pERK1 | 480 | 1.33 | pERK2 | 0 |
| 1.30 | pAkt | 240 | 1.32 | pIκBα | 15 |
| 1.28 | pIκBα | 480 | 1.32 | pJNK | 60 |
| 1.27 | pIκBα | 5 | 1.23 | pIκBα | 720 |
| 1.24 | pERK1 | 30 | 1.23 | pERK1 | 0 |
| 1.23 | pIκBα | 720 | 1.23 | pAkt | 15 |
| 1.23 | pIκBα | 90 | 1.21 | pERK1 | 15 |
| 1.22 | pERK1 | 120 | 1.21 | pERK2 | 720 |
| 1.19 | pERK1 | 720 | 1.20 | pIκBα | 5 |
| 1.19 | pERK1 | 60 | 1.19 | pIκBα | 60 |
| 1.18 | Clvd Casp-8 (MW = 43) | 720 | 1.18 | pIκBα | 480 |
| 1.16 | pIκBα | 120 | 1.16 | pIκBα | 240 |
| 1.12 | pERK1 | 240 | 1.12 | pERK1 | 30 |

[1]Variable importance of projection (4) .

**Supplementary Table S3.** Top 20 loadings in the first principal component of the apoptosis model

| $w_1c_1$ | Signal | Time (min) |
|---|---|---|
| 0.224 | pIκBα | 60 |
| 0.212 | pERK2 | 720 |
| 0.208 | pIκBα | 240 |
| 0.207 | pERK1 | 480 |
| 0.190 | pIκBα | 480 |
| 0.190 | pIκBα | 90 |
| 0.186 | pIκBα | 5 |
| 0.186 | pERK1 | 120 |
| 0.182 | pIκBα | 720 |
| 0.179 | pERK1 | 720 |
| -0.176 | pHSP27 | 0 |
| 0.175 | pIκBα | 120 |
| 0.169 | pERK1 | 240 |
| 0.164 | pIκBα | 15 |
| 0.162 | pERK2 | 240 |
| 0.161 | pERK2 | 480 |
| 0.159 | pERK1 | 60 |
| 0.156 | pERK1 | 30 |
| 0.147 | pAkt | 240 |
| 0.140 | pERK1 | 90 |

**Supplementary Table S4.** Top 20 loadings in the second principal component of the apoptosis model

| $w_2c_2$ | Signal | Time (min) |
|---|---|---|
| -0.330 | pJNK | 0 |
| -0.293 | pHSP27 | 0 |
| -0.292 | pJNK | 5 |
| 0.262 | pAkt | 240 |
| -0.255 | pAkt | 15 |
| -0.236 | pJNK | 720 |
| -0.235 | pHSP27 | 5 |
| -0.203 | pHSP27 | 720 |
| -0.201 | pJNK | 480 |
| -0.200 | pJNK | 90 |
| -0.177 | pERK2 | 5 |
| -0.168 | pHSP27 | 15 |
| -0.167 | pHSP27 | 480 |
| -0.164 | pJNK | 15 |
| -0.159 | pJNK | 60 |
| -0.158 | pERK1 | 5 |
| -0.154 | pERK2 | 0 |
| -0.153 | pERK1 | 0 |
| 0.153 | pERK1 | 30 |
| 0.147 | pIκBα | 0 |

**Supplementary Table S5.** Top 20 loadings in the third principal component of the apoptosis model

| $w_3c_3$ | Signal | Time (min) |
|---|---|---|
| -0.250 | pAkt | 90 |
| -0.249 | pAkt | 720 |
| 0.208 | pHSP27 | 240 |
| 0.207 | pERK1 | 30 |
| -0.204 | pAkt | 120 |
| -0.199 | pAkt | 60 |
| 0.199 | pHSP27 | 120 |
| 0.186 | pERK1 | 60 |
| 0.185 | pHSP27 | 90 |
| -0.178 | pAkt | 480 |
| 0.173 | pJNK | 240 |
| -0.167 | pAkt | 240 |
| -0.167 | pAkt | 5 |
| -0.165 | pAkt | 0 |
| -0.162 | pERK2 | 0 |
| 0.160 | pJNK | 120 |
| -0.154 | pERK1 | 0 |
| 0.153 | pERK2 | 60 |
| -0.153 | pIκBα | 5 |
| -0.151 | pAkt | 30 |

**Supplementary Table S6.** Top 20 loadings in the first principal component of the CXCL1/CXCL8 model

| $w_1c_1$ | Signal | Time (min) |
|---|---|---|
| 0.204 | pJNK | 15 |
| 0.201 | pIκBα | 120 |
| 0.195 | pJNK | 30 |
| 0.194 | pHSP27 | 15 |
| 0.194 | pIκBα | 90 |
| 0.193 | pHSP27 | 30 |
| 0.192 | pIκBα | 15 |
| 0.192 | pHSP27 | 60 |
| 0.190 | pJNK | 60 |
| 0.185 | pERK1 | 15 |
| 0.182 | pERK2 | 0 |
| 0.181 | pIκBα | 5 |
| 0.179 | pIκBα | 720 |
| 0.178 | pERK2 | 720 |
| 0.172 | pIκBα | 480 |
| 0.171 | pIκBα | 60 |
| 0.163 | pIκBα | 240 |
| 0.162 | pERK2 | 15 |
| 0.160 | pERK1 | 0 |
| 0.153 | pERK1 | 720 |

**Supplementary Table S7.** Top 20 loadings in the second principal component of the CXCL1/CXCL8 model

| $w_2c_2$ | Signal | Time (min) |
|---|---|---|
| 0.244 | pERK1 | 30 |
| -0.236 | pHSP27 | 0 |
| -0.223 | pAkt | 15 |
| -0.208 | pJNK | 5 |
| -0.207 | pAkt | 480 |
| -0.202 | pAkt | 5 |
| -0.201 | pAkt | 0 |
| -0.196 | pJNK | 0 |
| -0.184 | pAkt | 30 |
| 0.184 | Clvd Casp 8 (MW = 41) | 480 |
| 0.183 | pERK1 | 60 |
| 0.172 | pHSP27 | 240 |
| -0.169 | pERK1 | 0 |
| -0.168 | pHSP27 | 5 |
| -0.162 | pAkt | 60 |
| 0.159 | pHSP27 | 120 |
| -0.158 | pHSP27 | 720 |
| -0.157 | pERK2 | 0 |
| -0.155 | pERK2 | 5 |
| 0.148 | pERK1 | 120 |

**Supplementary Table S8.** Top 20 loadings in the third principal component of the CXCL1/CXCL8 model

| $w_3c_3$ | Signal | Time (min) |
|---|---|---|
| -0.247 | pERK2 | 90 |
| -0.233 | Clvd Casp 8 (MW = 43) | 480 |
| -0.224 | pERK1 | 5 |
| 0.223 | pHSP27 | 60 |
| 0.219 | pJNK | 30 |
| 0.218 | pIκBα | 30 |
| 0.212 | pHSP27 | 30 |
| 0.201 | pIκBα | 15 |
| -0.195 | pAkt | 120 |
| 0.191 | pJNK | 60 |
| -0.187 | pERK2 | 120 |
| 0.181 | pIκBα | 90 |
| -0.181 | pHSP27 | 5 |
| -0.178 | pERK2 | 5 |
| 0.174 | pIκBα | 720 |
| -0.173 | pERK1 | 90 |
| -0.163 | pJNK | 480 |
| 0.156 | pIκBα | 240 |
| -0.154 | pHSP27 | 480 |
| 0.152 | pIκBα | 120 |

## Supplemental References

1. Balkwill F. Tumour necrosis factor and cancer. Nat Rev Cancer 2009; 9: 361-71.
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3. Owens DM, Keyse SM. Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. Oncogene 2007; 26: 3203-13.
4. Gaudet S, Janes KA, Albeck JG, Pace EA, Lauffenburger DA, Sorger PK. A compendium of signals and responses triggered by prodeath and prosurvival cytokines. Mol Cell Proteomics 2005; 4: 1569-90.