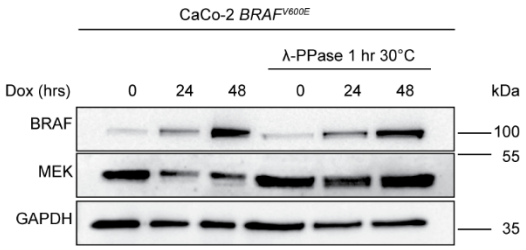
Discrete cytosolic macromolecular BRAF complexes exhibit distinct activities and composition

Britta Diedrich[1,2,#], Kristoffer T.G. Rigbolt[1,2,#], Michael Röring[3,#], Ricarda Herr[3], Stephanie Kaeser-Pebernard[8], Christine Gretzmeier[1,2,4], Robert F. Murphy[4,5], Tilman Brummer[2,3,6,7,§] and Jörn Dengjel[1,2,4,6,8,§]

Appendix

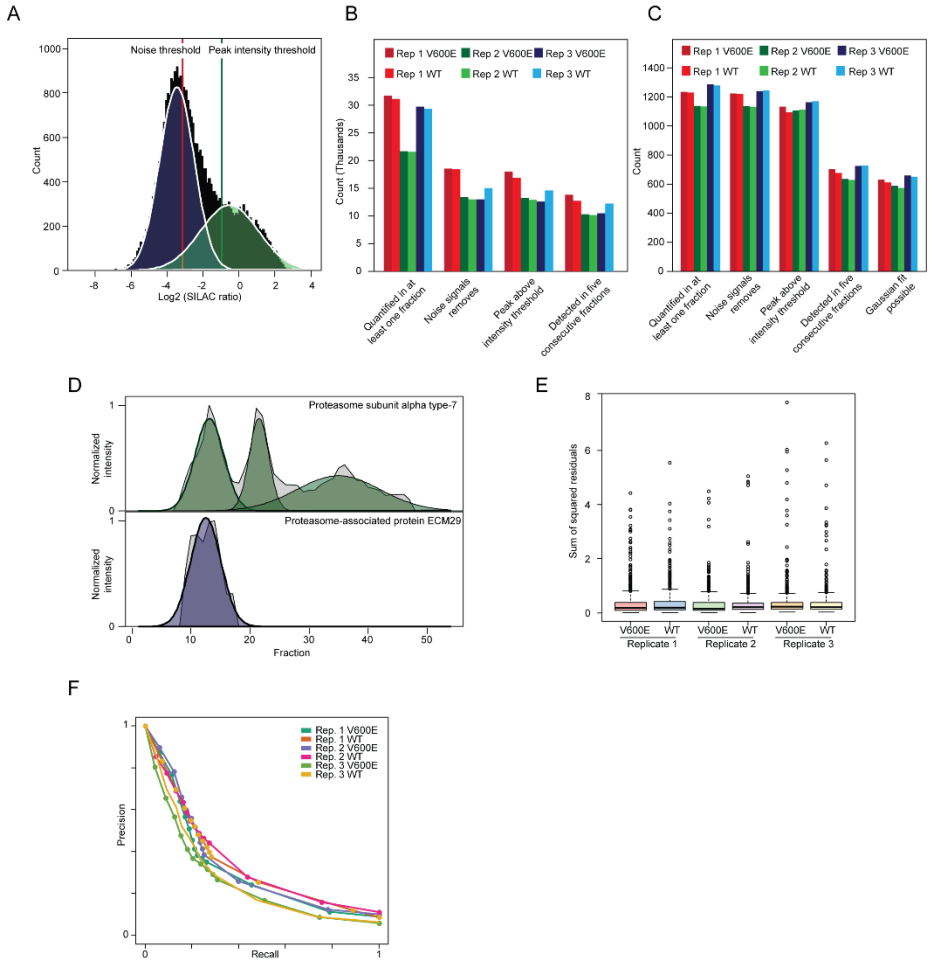
- Appendix Figure S1: MEK protein level analysis.
- Appendix Figure S2: SEC-PCP-SILAC data processing.
- Appendix Figure S3: Molecular weight analysis by SEC.
- Appendix Figure S4: SILAC-based analysis of BRAF protein interactions.
- Appendix Figure S5: Time course of 17-AAG treatment.
- Appendix Figure S6: BRAF complexes exhibit differential activities.

Appendix Figure S1



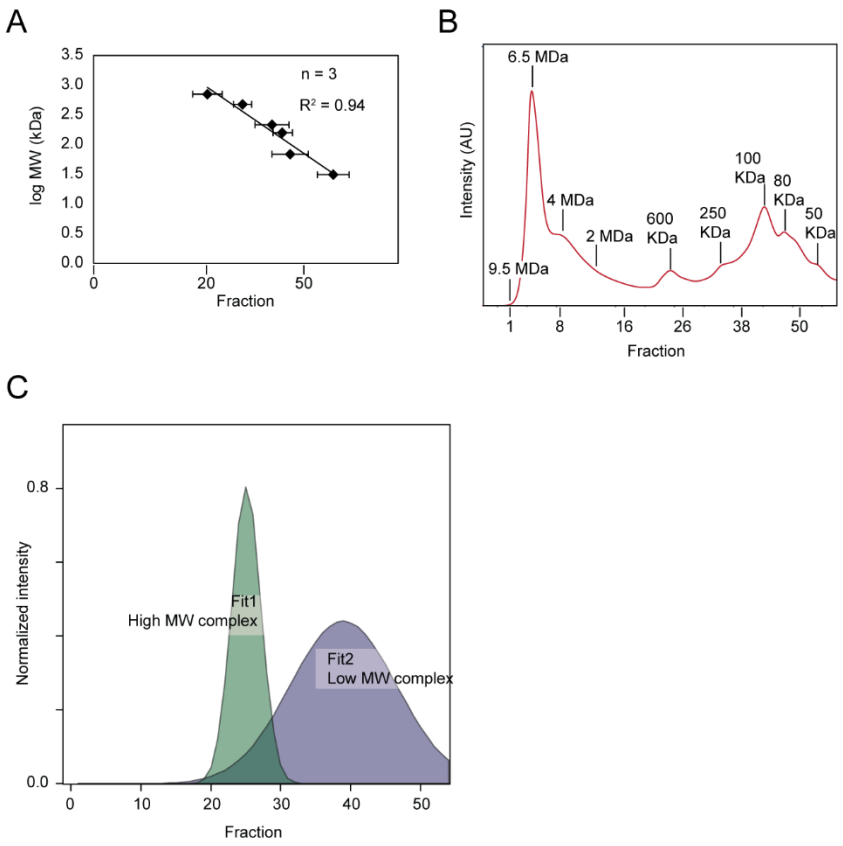
Appendix Figure S1. MEK protein level analysis. Dox induced *BRAF*^{V600E} expression leads to an increase in MEK phosphorylation (see Figure 1), which reduces reactivity of the anti-MEK antibody. One h of λ-phosphatase treatment (400 U) reduces phosphorylation and recovers anti-MEK reactivity.

Appendix Figure S2



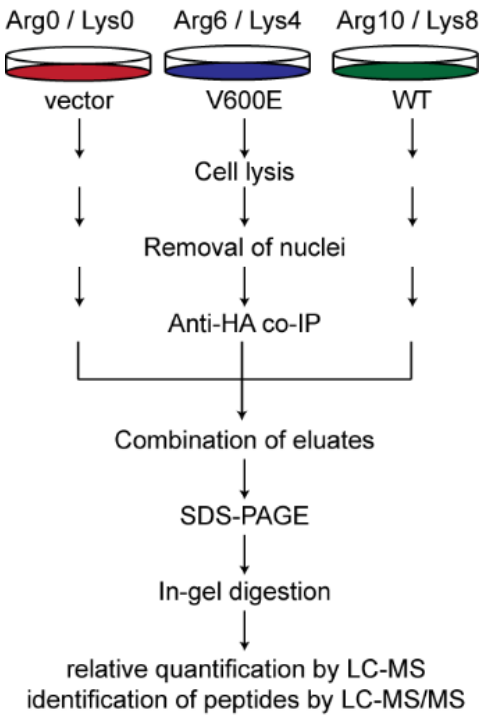
Appendix Figure S2: SEC-PCP-SILAC data processing. (A) Distribution of all recorded SILAC ratios with the two fitted Gaussian distributions overlaid with the blue distribution presumably containing predominantly noise signals and the green containing the useful data of protein elution profiles. The red line indicates the threshold below which all ratios are regarded as noise and are removed. The green line indicates the value of which all proteins must contain at least one equal or higher to be regarded as sufficiently free of the noise. **(B)** Number of remaining peptide ratios and **(C)** protein ratios after the individual filtering steps. **(D)** Example of the principle behind the use of fitted Gaussian curves for protein interaction inference. On top the elution of Proteasome subunit alpha type-7 with three overlaid Gaussian curves is shown and it is observed that only the leftmost curve overlaps with the proteasome associated protein ECM29. **(E)** Boxplots of the residuals between the raw chromatograms and the fitted curves. All fits giving rise to a sum of squared residuals indicated as outliers were discarded. **(F)** Precision-recall curves of the three replicates used to obtain a threshold for considering two proteins as sufficiently similar in elution to be regarded as potential interactors.

Appendix Figure S3



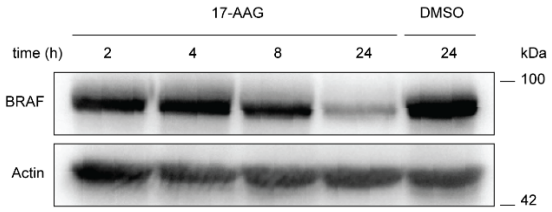
Appendix Figure S3: Molecular weight analysis by SEC. (A) Analysis of molecular weight standards by SEC. Error bars indicate standard deviation. **(B)** Representative SEC profile of Caco2tet cell cytosol. Estimated molecular weights are indicated. **(C)** The Gaussian fits of the elution profile of $BRAF^{V600E}$, compare with Figure 3B.

Appendix Figure S4



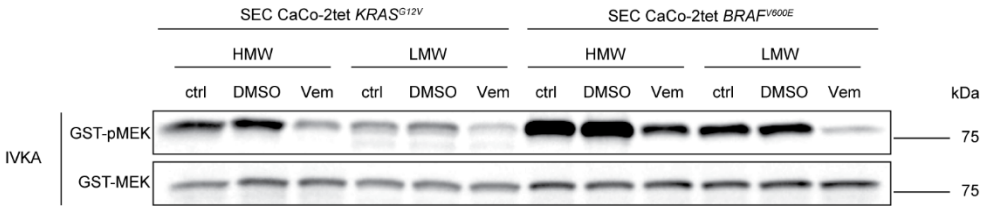
Appendix Figure S4: SILAC-based analysis of BRAF protein interactions. SILAC labeling strategy and MS-based proteomics workflow to study differences in protein-protein interactions of $BRAF^{WT}$ and $BRAF^{V600E}$.

Appendix Figure S5



Appendix Figure S5: Time course of 17-AAG treatment. Time response of CaCo-2tet *HA-BRAF*^{V600E} cells to 1 µM 17-AAG after 48 h of doxycyclin induction. After 8-24 h, a reduction in BRAF amount is observed.

Appendix Figure S6



Appendix Figure S6: BRAF complexes exhibit differential activities. The high and low molecular weight complexes of WT and V600E are fractionated by SEC and kinase activities are analyzed by *in vitro* kinase assays (IVKA). The phosphorylation of purified GST-MEK is detected by a phosphosite-specific antibody recognizing phospho-Ser217/221 (pMEK). Ctrl is without DMSO and Vemurafenib (Vem), Vem is dissolved in 100% DMSO, Vem and DMSO are diluted 1:10,000 in medium. Vem final conc. = 1 µM.