Functional genomic characterization of a synthetic anti-HER3 antibody reveals a role for ubiquitination by RNF41 in the anti-proliferative response

**Jacob P. Turowec, Esther W. T. Lau, Xiaowei Wang, Kevin R. Brown, Frederic A. Fellouse, Kamaldeep K. Jawanda, James Pan[#], Sachdev S. Sidhu[$]\*, Jason Moffat[$]\***

Supporting Information Materials:

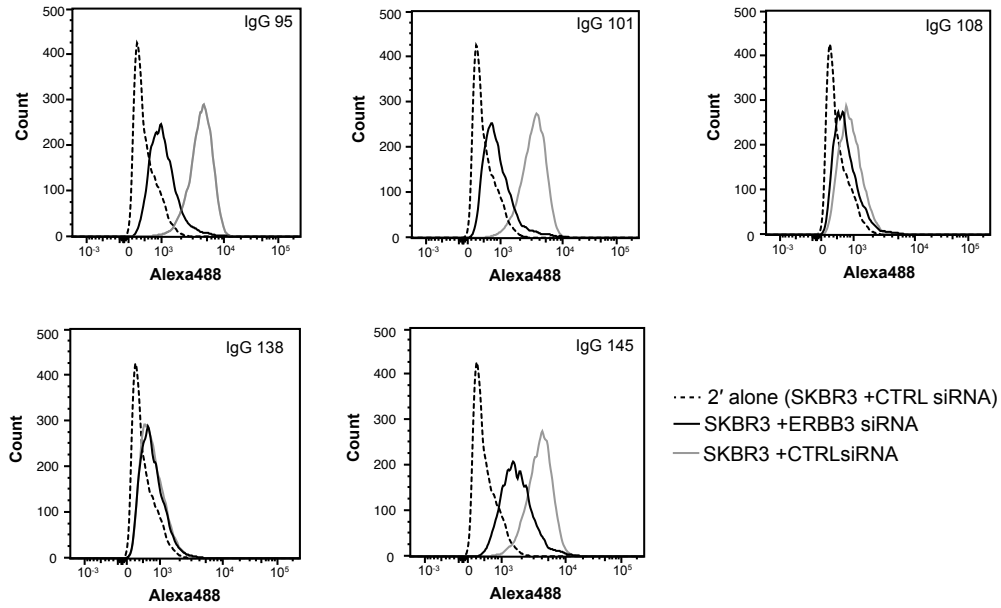**Supplemental Figure 1.** FACS with anti-HER3 IgGs

**Supplemental Figure 2.** Genomic shRNA screen for desensitization to IgG 95

**Supplemental Figure 3.** BxPC3 cells respond to IgG 95 in low serum conditions
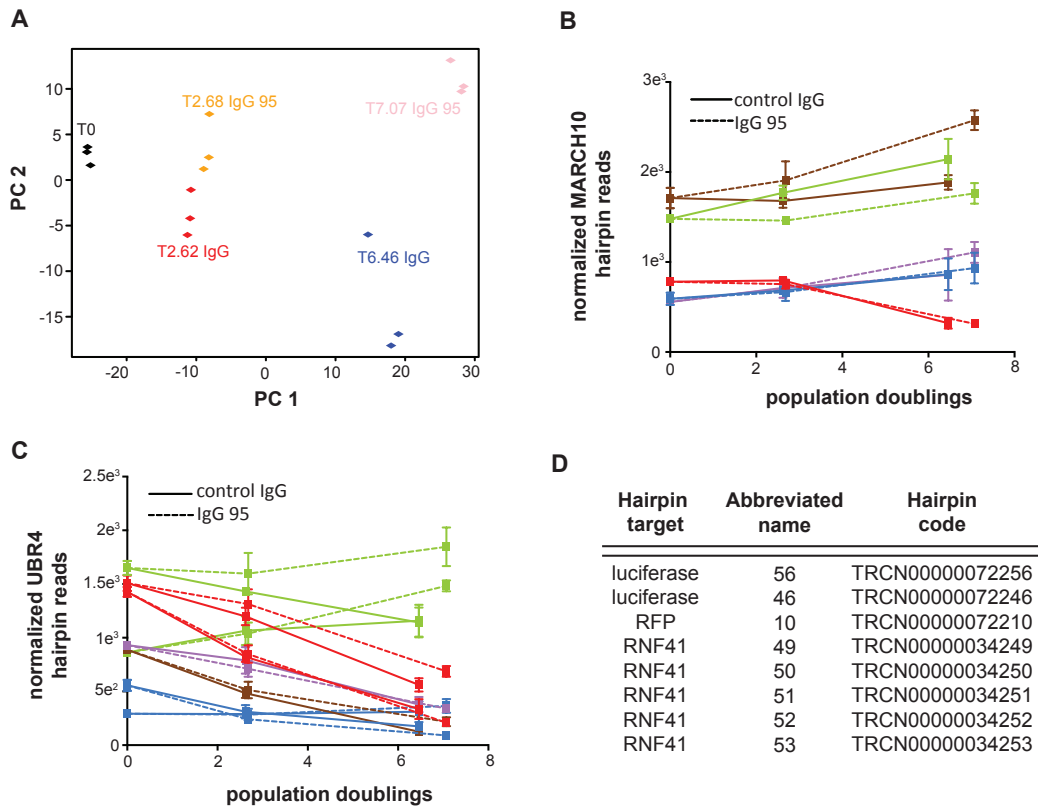
**Table S1.** IP-MS of IgG 95

**Table S2.** List of shRNAs in UPS library
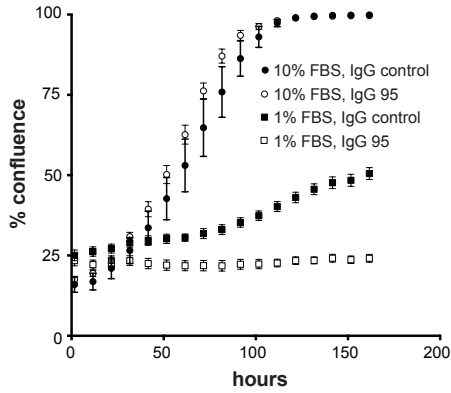
**Supplemental Figure 1**



**Supplemental Figure 1. FACS with anti-HER3 IgGs.** SKBR3 cells were transfected with control or HER3 siRNA and then stained with the indicated IgG, followed by anti-Fab fragment (human) Alexa-fluor 488, and flow cytometry analysis. The data is representative of 2 (IgG 108, 138, 145) or 3 (IgG 95, 101) independent experiments where at least 5000 cells were measured.

**Supplemental Figure 2**

A

B

C

D

| Hairpin target | Abbreviated name | Hairpin code |
|---|---|---|
| luciferase | 56 | TRCN00000072256 |
| luciferase | 46 | TRCN00000072246 |
| RFP | 10 | TRCN00000072210 |
| RNF41 | 49 | TRCN00000034249 |
| RNF41 | 50 | TRCN00000034250 |
| RNF41 | 51 | TRCN00000034251 |
| RNF41 | 52 | TRCN00000034252 |
| RNF41 | 53 | TRCN00000034253 |

**Supplemental Figure 2. Genomic shRNA screen for desensitization to IgG 95.** (A) A principal component analysis of the screen was performed and plotted to highlight divergence and clustering of the different treatment groups. (B) Normalized MARCH10 and (C) UBR4 hairpin reads from the screen. Counts from IgG control cell lines have a solid line, whereas IgG 95 treated samples are illustrated with a dashed line. (D) A list of the shRNA vectors used in validation studies.

**Supplemental Figure 3**



**Supplemental Figure 3. BxPC3 cells respond to IgG 95 in low serum conditions.** BxPC3 cells were seeded in normal (10%) or low (1%) FBS media, and treated the next day as indicated. Proliferation was monitored by measuring the % confluence with an Incucyte Zoom (Sartorius). Error bars represent the standard deviation of quadruplicate replicates grown in parallel. The data is representative of two independent experiments.