Supplementary Materials

GDF15 Mediates the Effect of Skeletal Muscle Contraction on Glucose-Stimulated Insulin Secretion

Hui Zhang, Anny Mulya, Stephan Nieuwoudt, Bolormaa Vandanmagsar, Ruth McDowell, Elizabeth C. Heintz, Elizabeth R.M. Zunica, J. Jason Collier, Nadejda Bozadjieva-Kramer, Randy J. Seeley, Christopher L. Axelrod, and John P. Kirwan

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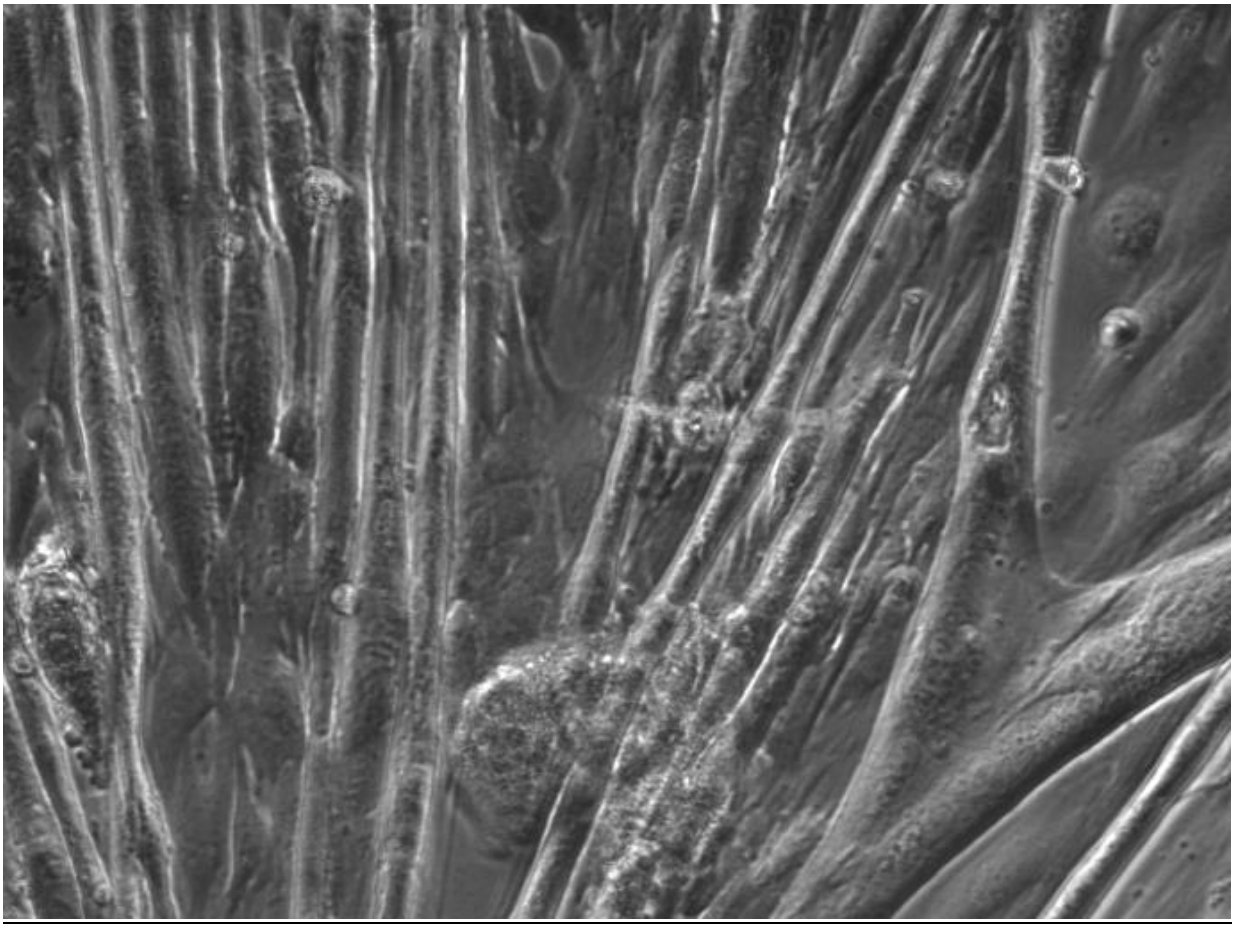
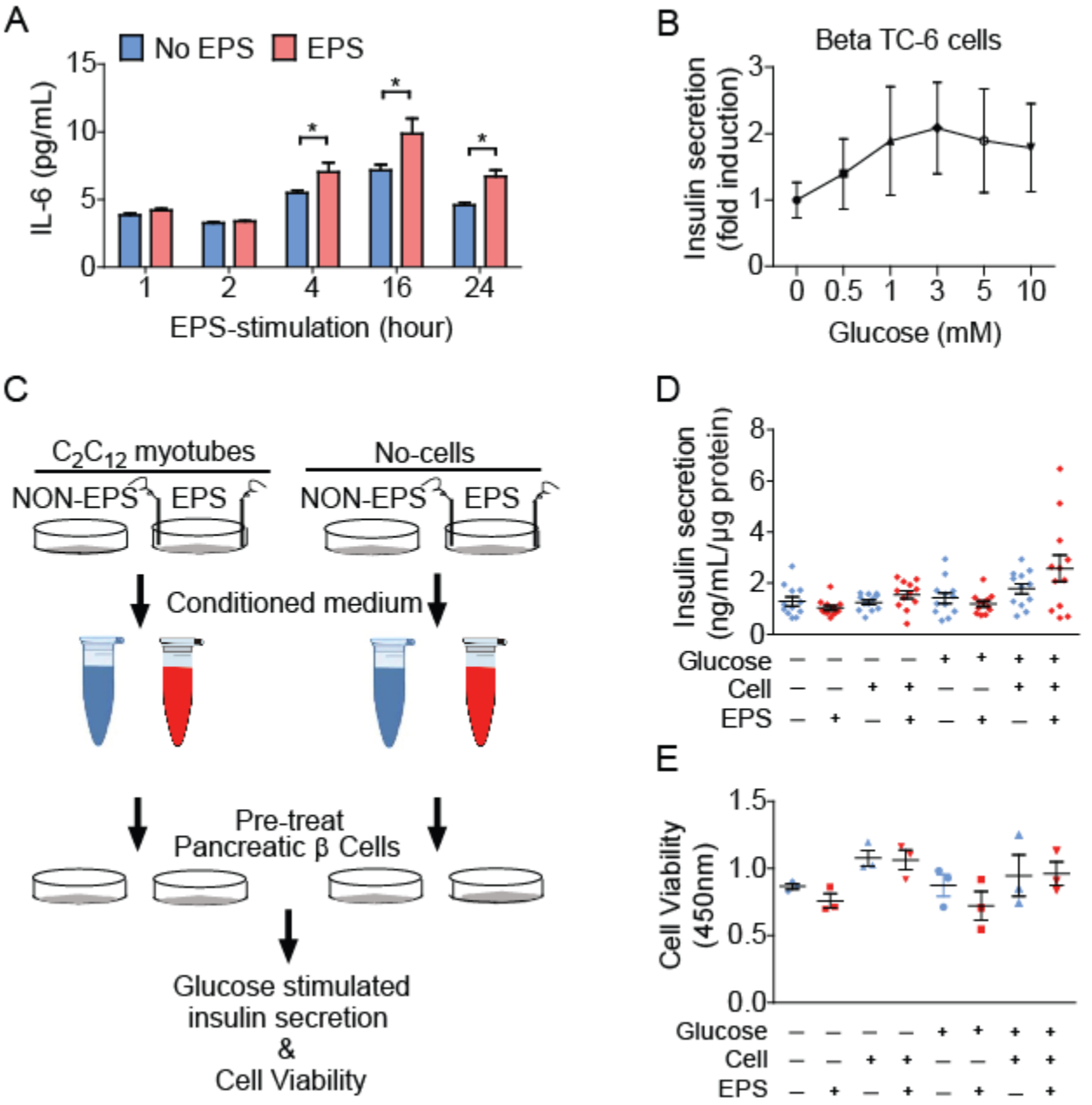
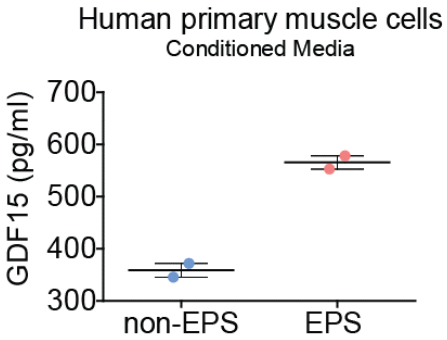


Supplementary Figure 1. Related to Figure 1. Video illustration of C2C12 myotube contraction after EPS-exposure for 16 hours.

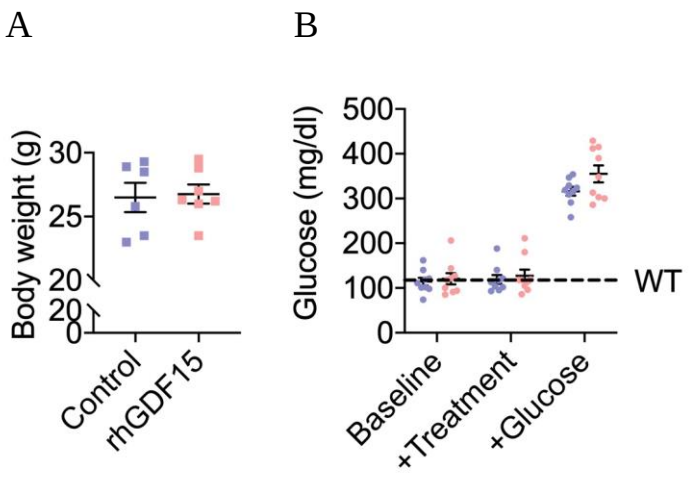


Supplementary Figure 2. Related to Figures 1 and 2. (A) IL-6 in the conditioned media from non-EPS and EPS exposed C2C12 myotubes determined by ELISA-assay (*n*=6). (B) Glucose stimulated insulin secretion in beta TC-6 cells (*n*=5 repeated culture, 4-5 replicates/culture) (C) Experimental flow to investigate a crosstalk between muscle contraction and pancreatic beta cell function. Myotubes (left) and only culture media as a cell-free control (right) were exposed to EPS (in red) or without EPS (in blue) for overnight. Then media from all 4 groups: myotube-generated non-EPS-CM and EPC-CM, and cell-free non-EPS and EPS, were applied to β-cells for overnight. Then insulin secretion was evaluated in β-cells with or without 1 mM glucose and cell viability was examined afterword. (D) Insulin secretion in β-TC-6 cells exposed to conditioned media from non-EPS and EPS-exposed C2C12 myotubes and cell-free non-EPS and EPS media (*n*=12). B-

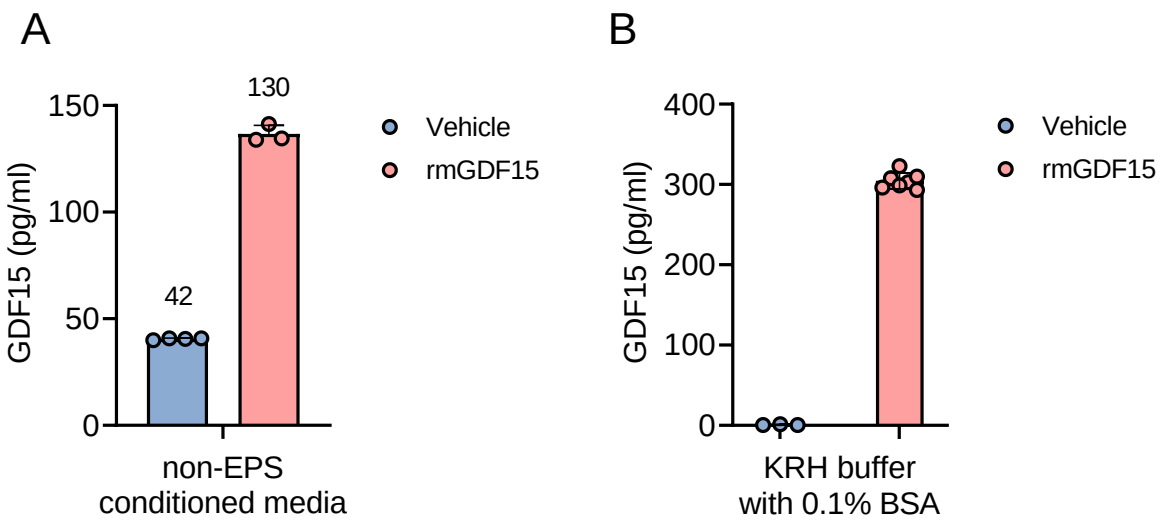
cells treated with EPS-exposed media are marked by red, while β-cells treated with non-EPS media are marked by blue. Treatment with cell-free media, but exposed to EPS without glucose (red, 2nd group on the graph) and with glucose (red, 6th group on the graph) did not cause increase in insulin secretion in β-cells. However, only myotubes-generated EPC-conditioned media in the presence of glucose (red, 8th group on the graph) increased insulin secretion (GSIS) in β-cells. (E) Effect of myoutubes-generated and cell-free EPS-conditioned media on the viability of β-cells (*n*=3*)*. Cell viability was not different in all 8 treatment conditions that were used for the insulin secretion assay in D. Data are expressed as mean±SE. **P*<0.05 compared to non-EPS by two-way ANOVA with Bonferroni post-hoc test.



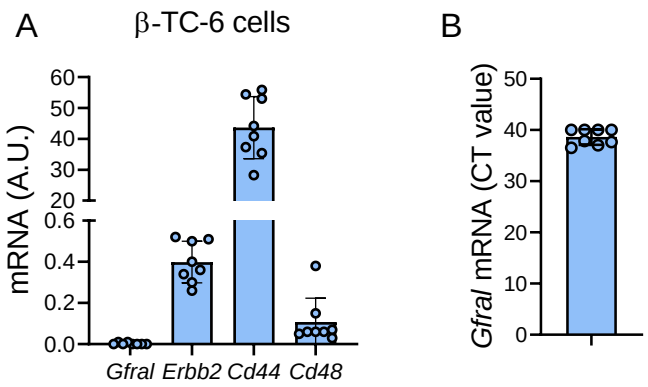
Supplementary Figure 3. Related to Figures 1 and 2. GDF15 protein secretion in the conditioned medium of EPS-stimulated human primary skeletal muscle cells (*n*=2).



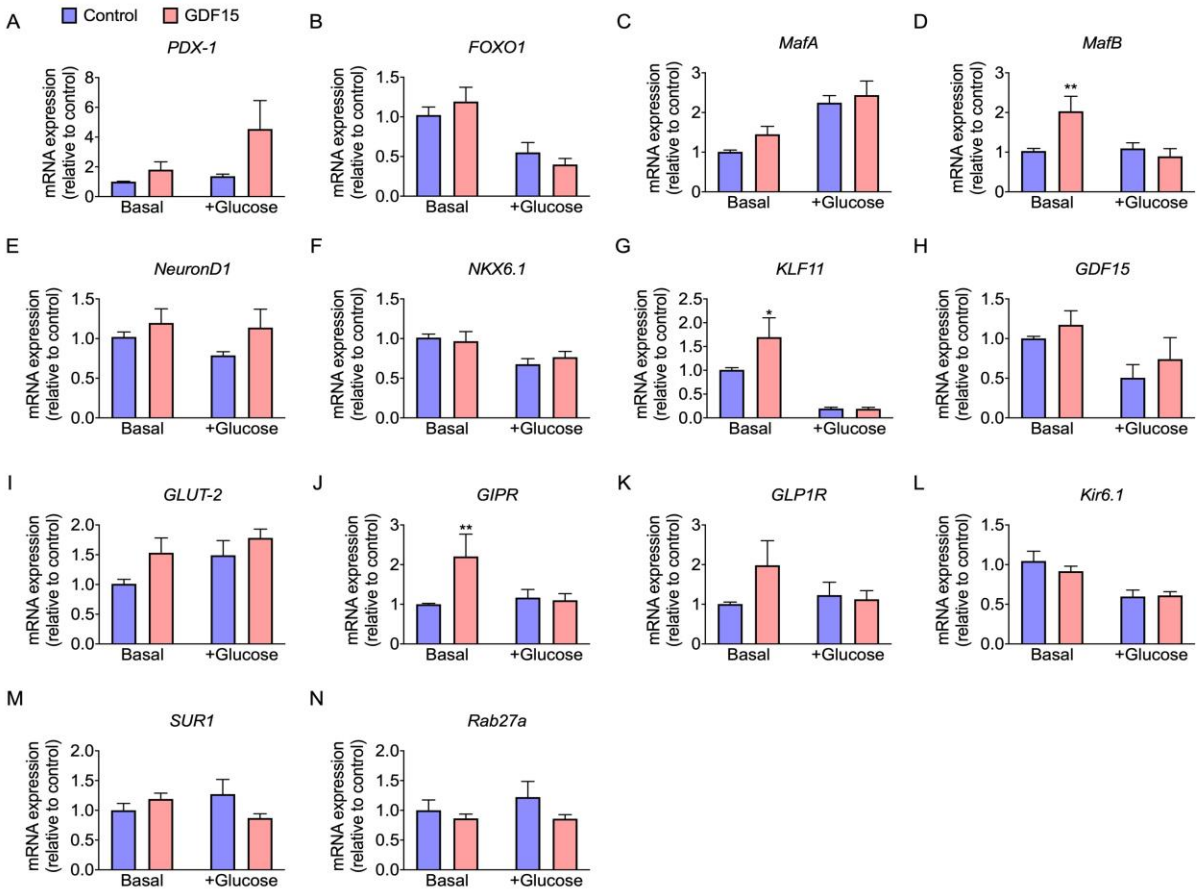
Supplementary Figure 4. Related to Figure 3. (A) Body weight of chow diet-fed C57BL/6 male mice injected saline or 0.25 mg/kg of rhGDF15 (*n*=6-7). (B) Plasma glucose levels C57BL/6 mice injected saline or rhGDF15 and 1g/kg of D-glucose (*n*=6-7).



Supplementary Figure 5. Related to Figures 3 and 4. (A) Mouse GDF15 levels in non-EPS conditioned media supplemented with vehicle or recombinant mouse GDF15 protein (*n*=3 repeated culture). (B) Mouse GDF15 levels in KRH buffer supplemented with vehicle or 300 pg/ml of rmGDF15 (n=3 repeated culture, 4-6 replicates/culture).

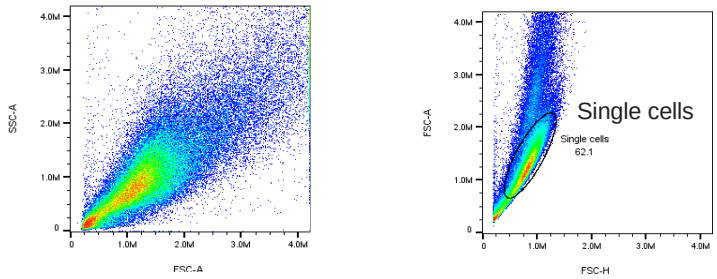


Supplementary Figure 6. Related to Figure 4. Gene expression of receptors that may regulate GDF15 action in beta cells. Relative gene expression was evaluated by qPCR in cultured beta-TC-6 cells and expressed in (A) as arbitrary unit and in (B) as CT value (*n*=8). Data are expressed as mean ± SD.

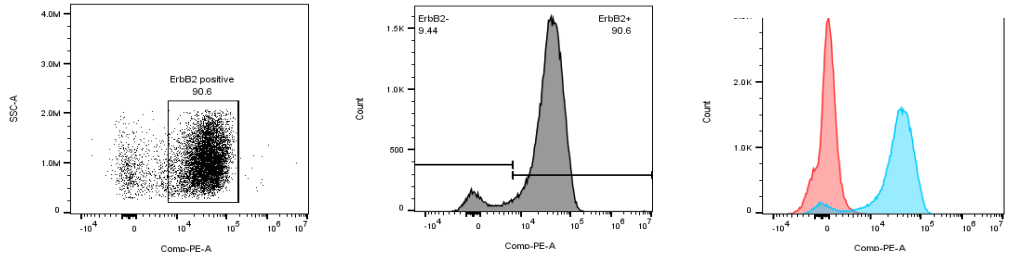


Supplementary Figure 7. Related to Figure 5. Expression of genes involved in maintaining insulin secretion. Beta TC-6 cells were treated with vehicle or rmGDF15. Gene expression was evaluated by qPCR at baseline (without glucose) and glucose-stimulated states (*n*=6).

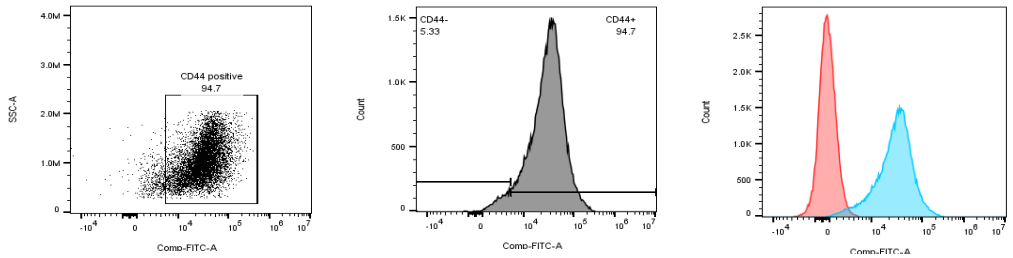
A



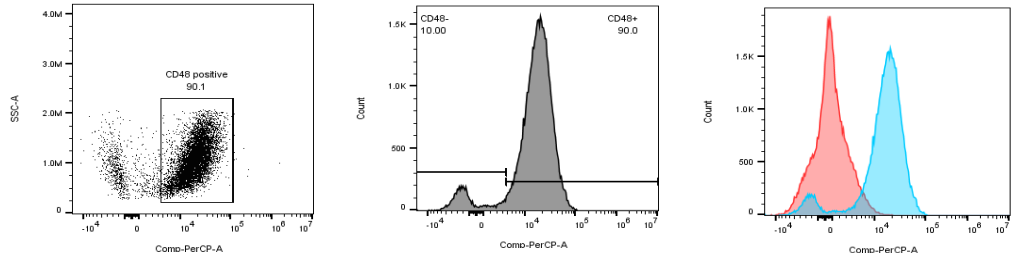
B ErbB2-PE



C CD44-FITC



D CD48-PerCP



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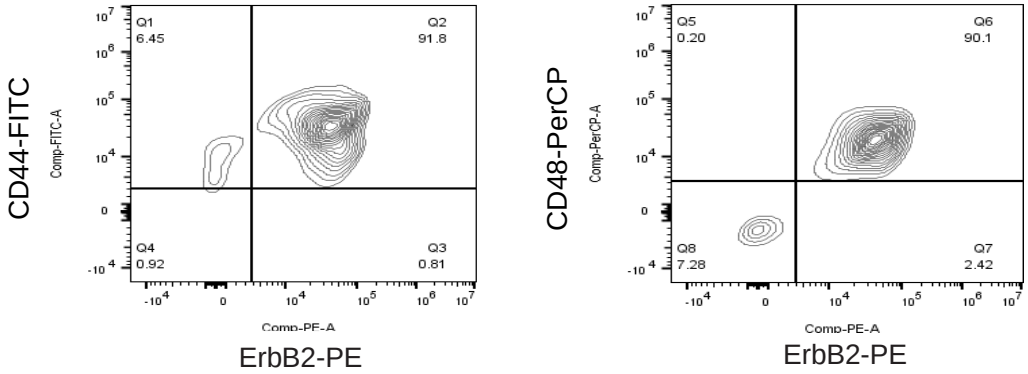

Supplementary Figure 8. Related to Figures 4 and 5. Flow cytometry images of beta-TC-6 cells stained for surface receptors ErbB2, CD44 and CD48. (A) Forward (FSC) vs Side (SSC) scatter plot of beta cells (left) and single cell population determined on the plot FSC-H vs FSC-A (right). Single cell population of beta cells was further gated on PE, FITC and PerCP to acquire beta cells positive for ErbB2 (B), CD44 (C) and CD48 (D). Positively stained beta cell numbers and images are shown in dot plot (left), histogram (center) and layout (right) where positive cells (in blue) overlaid over unstained negative control cells (in pink). (E) Counter plot images of double positive cells ErbB2^{+}CD44^{+} (left) and ErbB2^{+}CD48^{+} (right) in Q2 quadrants.