(**Fig. 1c**)). Finally, exposing HEK cells expressing αGFP-TRPV1/GFP-ferritin to radio waves (465 kHz) significantly increased intracellular calcium compared with what was seen in nontransfected controls (2.9-fold versus 0.8-fold change in Fluo-4 fluorescence, **Fig. 1d**).

Next, we tested the efficiency of the three constructs in transducing an RF signal into gene expression *in vitro* using a synthetic calcium-responsive reporter gene. As previously reported[9], this Ca^{++}-sensitive promoter is comprised of a 5′ regulatory region of three serum

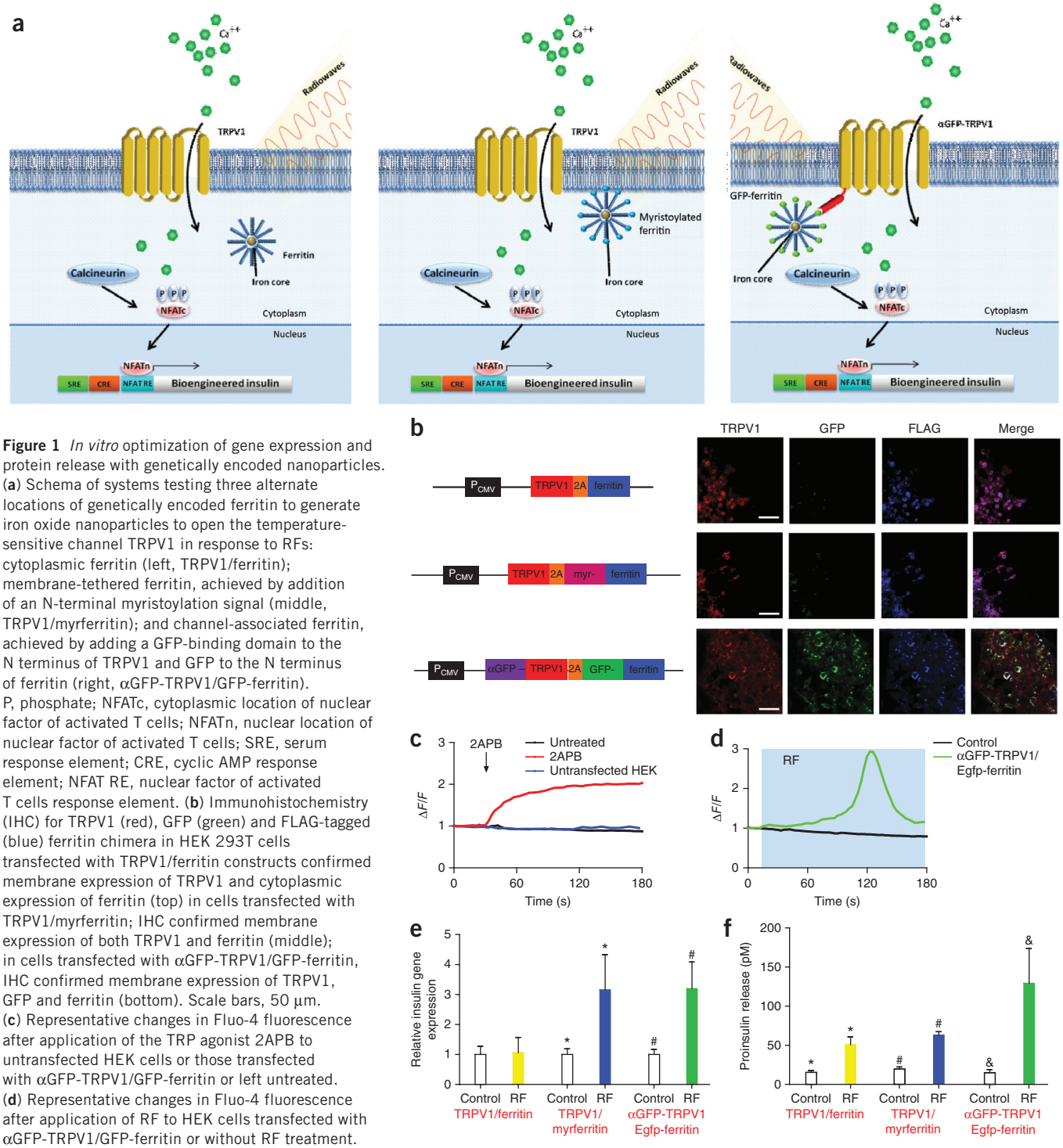

Figure 1 *In vitro* optimization of gene expression and protein release with genetically encoded nanoparticles. (**a**) Schema of systems testing three alternate locations of genetically encoded ferritin to generate iron oxide nanoparticles to open the temperature-sensitive channel TRPV1 in response to RFs: cytoplasmic ferritin (left, TRPV1/ferritin); membrane-tethered ferritin, achieved by addition of an N-terminal myristoylation signal (middle, TRPV1/myrferritin); and channel-associated ferritin, achieved by adding a GFP-binding domain to the N terminus of TRPV1 and GFP to the N terminus of ferritin (right, αGFP-TRPV1/GFP-ferritin). P, phosphate; NFATc, cytoplasmic location of nuclear factor of activated T cells; NFATn, nuclear location of nuclear factor of activated T cells; SRE, serum response element; CRE, cyclic AMP response element; NFAT RE, nuclear factor of activated T cells response element. (**b**) Immunohistochemistry (IHC) for TRPV1 (red), GFP (green) and FLAG-tagged (blue) ferritin chimera in HEK 293T cells transfected with TRPV1/ferritin constructs confirmed membrane expression of TRPV1 and cytoplasmic expression of ferritin (top) in cells transfected with TRPV1/myrferritin; IHC confirmed membrane expression of both TRPV1 and ferritin (middle); in cells transfected with αGFP-TRPV1/GFP-ferritin, IHC confirmed membrane expression of TRPV1, GFP and ferritin (bottom). Scale bars, 50 μm. (**c**) Representative changes in Fluo-4 fluorescence after application of the TRP agonist 2APB to untransfected HEK cells or those transfected with αGFP-TRPV1/GFP-ferritin or left untreated. (**d**) Representative changes in Fluo-4 fluorescence after application of RF to HEK cells transfected with αGFP-TRPV1/GFP-ferritin or without RF treatment. (**e**) RF treatment increases insulin gene expression in HEK cells expressing TRPV1/myrferritin and αGFP-TRPV1/GFP-ferritin but not in those expressing TRPV1/ferritin. Each study was repeated on 2 (TRPV1/ferritin) or 3 occasions (TRPV1/myrferritin and GFP-TRPV1/GFP-ferritin) each with 4 replicates (see Online Methods). In all cases, columns represent mean and error bars show s.e.m. Data were analyzed by a two-tailed Mann-Whitney test. * and # indicate $P < 0.05$. (**f**) RF treatment increases proinsulin release from HEK cells expressing TRPV1/ferritin, TRPV1/myrferritin and αGFP-TRPV1/GFP-ferritin. Each study was repeated on 2 (TRPV1/ferritin) or 3 occasions (TRPV1/myrferritin and αGFP-TRPV1/GFP-ferritin) each with 4 replicates. In all cases, columns represent mean and error bars indicate s.e.m. Data were analyzed by a two-tailed Mann-Whitney test. *, # and & indicate $P < 0.05$.