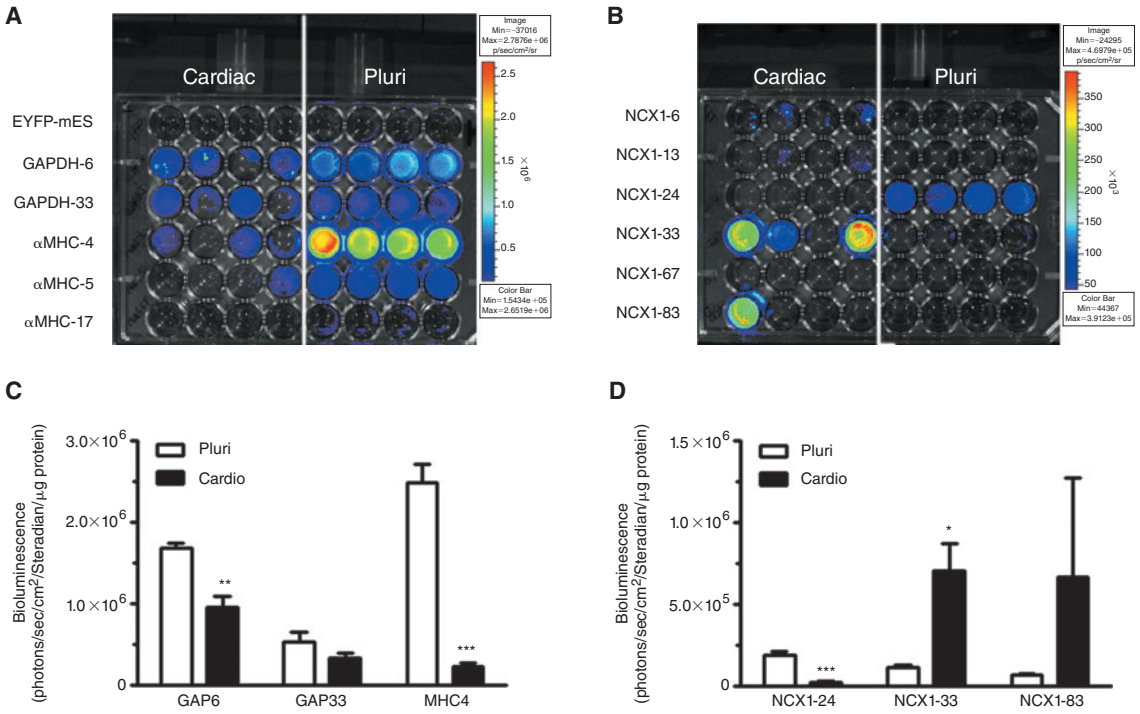
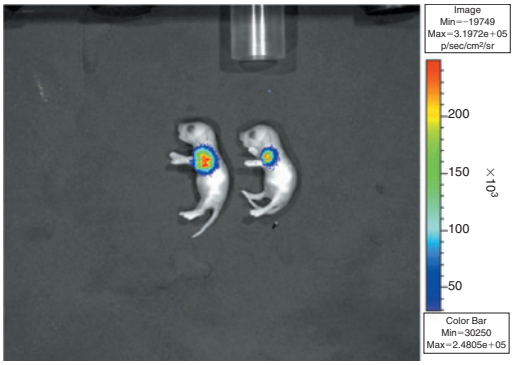
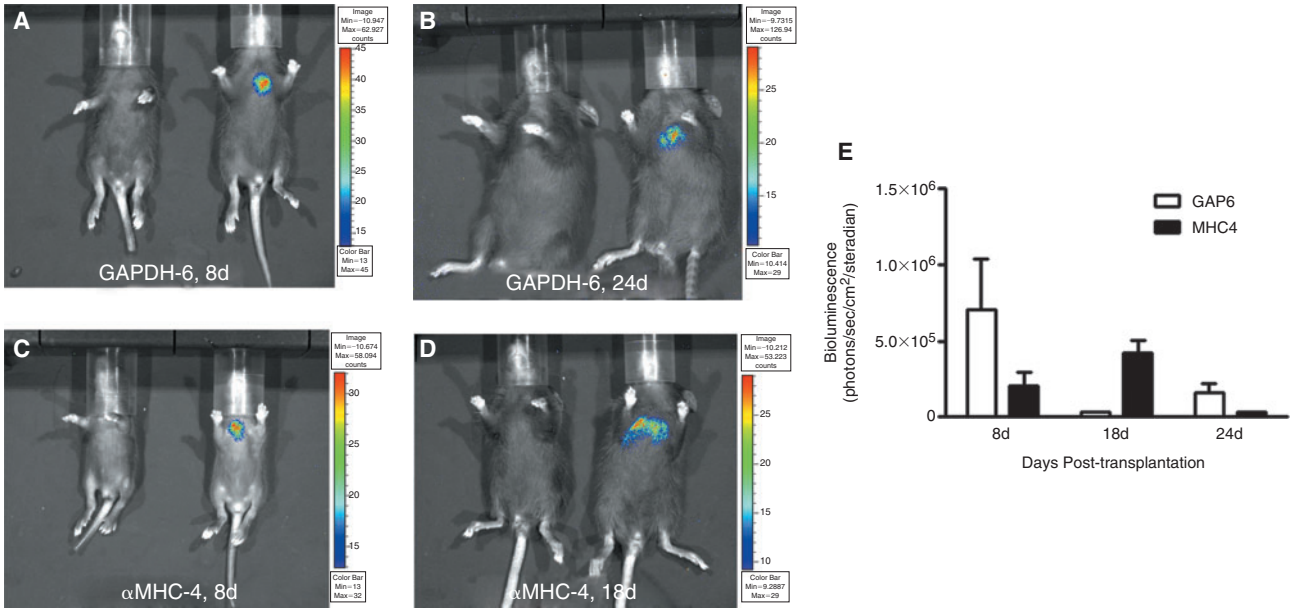


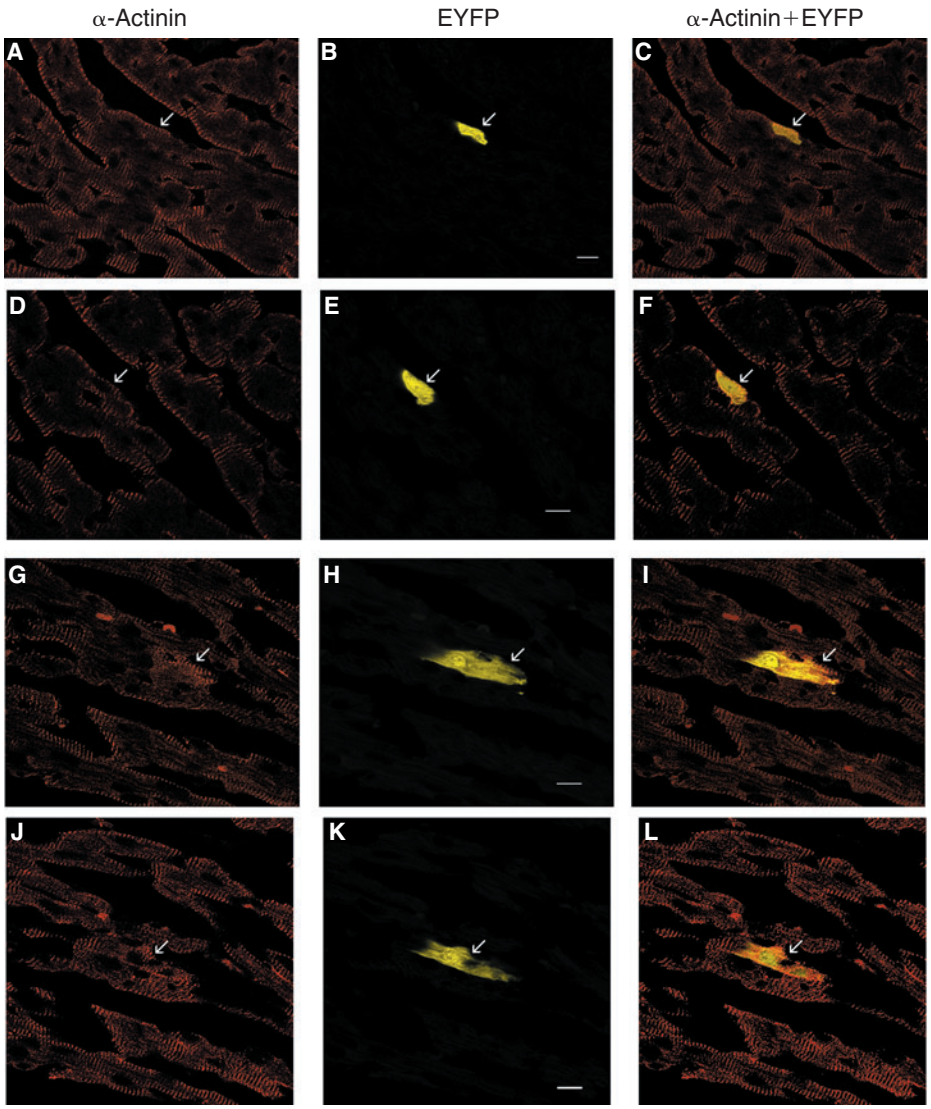
SUPPLEMENTARY FIG. 1. Secondary screening of G418-resistant clones: Pluripotent (pluri) versus cardiac-differentiated (cardio) mouse embryonic stem (mES) cells. (**A**) Various recombinant mES clones are listed for each row. Row 1, parental (control) enhanced yellow fluorescent protein (EYFP) mES cells. Rows 2–3, *GAPDH*-LUC clones #6 and #33. Rows 4–6, *α-MHC*-LUC clones #4, #5, and #17. (**B**) *Ncx-1*-LUC clones. In both cases, cardiac differentiation was induced using the hanging-drop method, and 6 embryoid bodies (EBs) per well were seeded into 4 wells per clone on the left side of each 48-well plate. Beating activity developed within 1–2 days after seeding. Pluripotent (undifferentiated) mES cells for each clonal line were seeded into the 4 wells (5×10^4 cells/well) on the right side of each plate in the same row as the corresponding EBs for that clone (indicated to the left of each row). Quantitative assessment of the luciferase (LUC) enzyme results normalized to total protein per well are shown graphically in panels **C** and **D**. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ for comparisons between pluri and cardio cells from each clonal line ($n = 4$ each).



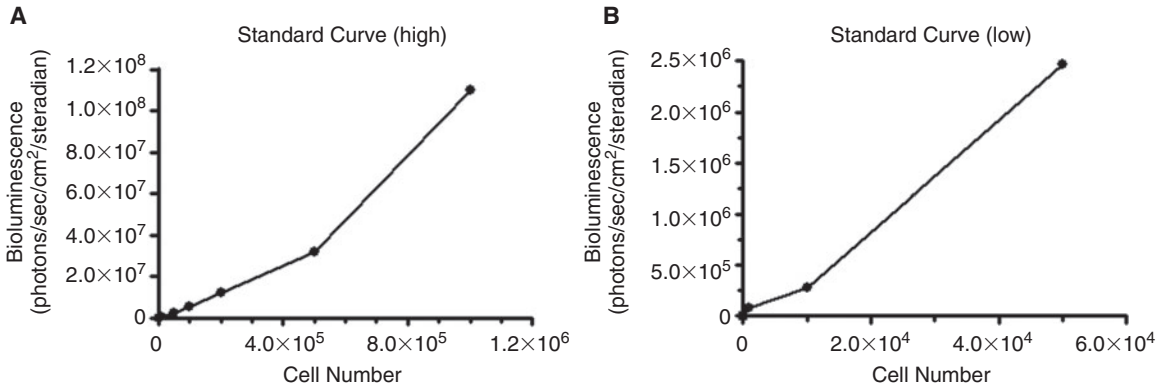
SUPPLEMENTARY FIG. 2. In vivo bioluminescence imaging (BLI) of transplanted pluripotent mES cells. Neonatal mouse hearts were injected with 100,000 undifferentiated *α-MHC*-4LUC and imaged 5 min later as shown. The pups were then returned to their mother where they appeared to recover normally. There was remarkably low incidence of mortality associated with procedure as we lost only 1 or 2 pups out of >100 that have been injected using this procedure.



SUPPLEMENTARY FIG. 3. In vivo BLI of transplanted *GAPDH*-6LUC and *α-MHC*-4LUC mES cells. For these experiments, 100,000 undifferentiated mES cells were injected into the neonatal mouse heart and imaged at 8, 18, and 24 days. Representative mice from different ages are shown for each cell line. (**A** and **B**) *GAPDH*-6LUC mES cells at 8 and 24 days post-transplantation. (**C** and **D**) α-MHC-4LUC mES cells at 8 and 18 days post-transplantation. (**E**) Graphical depiction of in vivo BLI activity for the indicated clones over the 24 days time course assessed for this experiment.



SUPPLEMENTARY FIG. 4. Dual fluorescent labeling of sarcomeric α-actinin and EYFP in heart sections from mice that had received an injection of 50,000 cardiac-differentiated *Ncx-1*-43LUC cells per heart. Within each row of panels, the same heart section is stained for sarcomeric α-actinin (muscle cells) or EYFP (mES cells) as shown in the leftmost and middle columns, respectively. The rightmost column displays an overlay of the α-actinin and EYFP staining. An arrow indicates cells that co-express α-actinin and EYFP in each row set. Scale bar, 10 μm.



SUPPLEMENTARY FIG. 5. Standard curves showing the relationship between BLI and cell number. Pluripotent *Ncx-1*-43LUC cells were seeded into 24-well Petri dishes at various concentrations per well (in 1 mL of culture medium): 0, 1,000, 10,000, 50,000, 100,000, 200,000, 500,000, and 1,000,000 cells. The cells were allowed to settle and attach over a 4-h incubation period. They were then evaluated for LUC activity using the Promega Bright-Glo Assay. Light production was measured by BLI using the IVIS, and confirmed using scintillation counting. (**A**) Standard curve for the high concentrations of cells. (**B**) Standard curve for the low concentrations of cells.