

## Supplementary Information files

### Title:

**Myocyte-specific enhancer factor 2c triggers transdifferentiation of adipose tissue-derived stromal cells into spontaneously beating cardiomyocyte-like cells**

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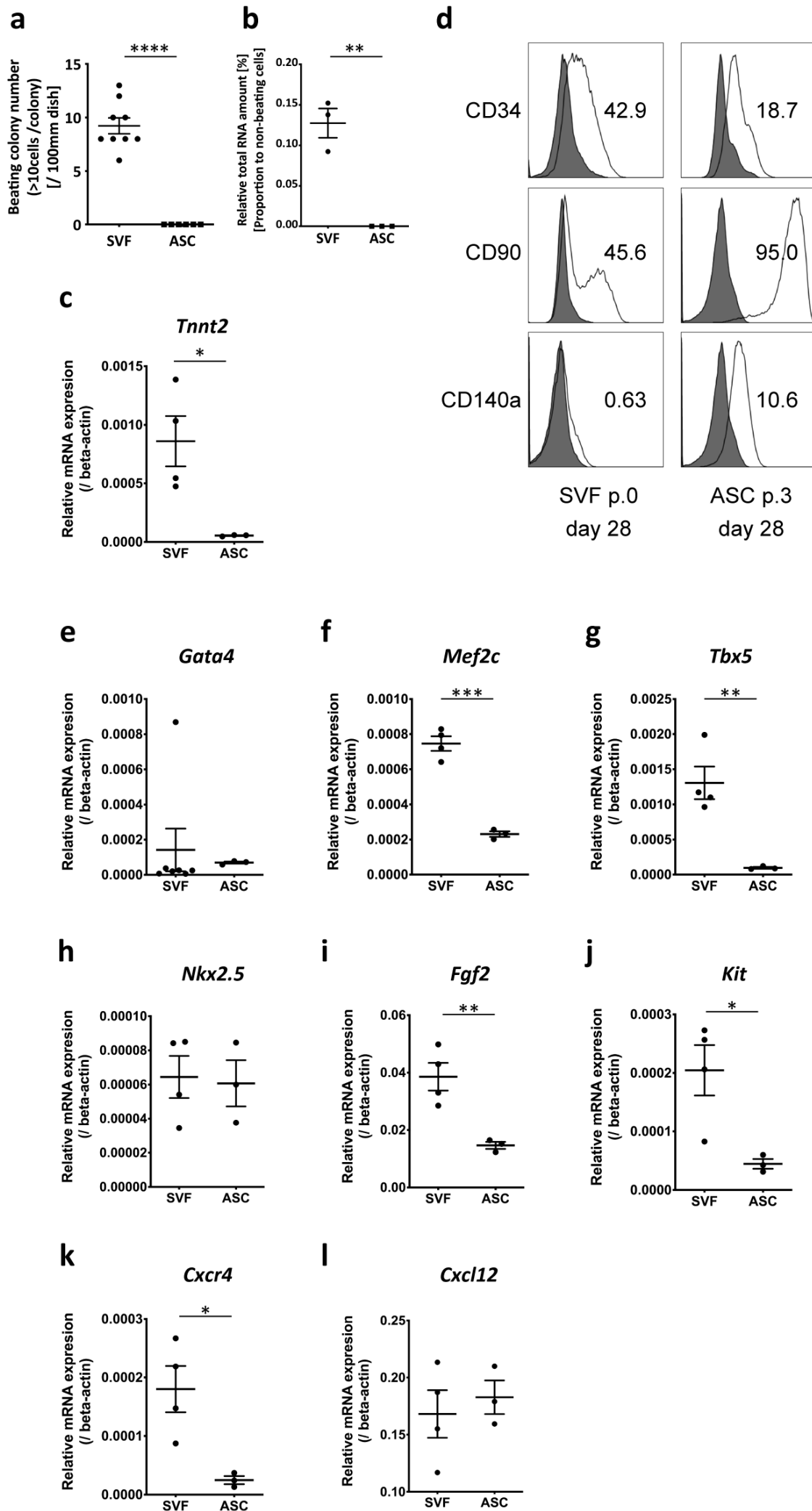
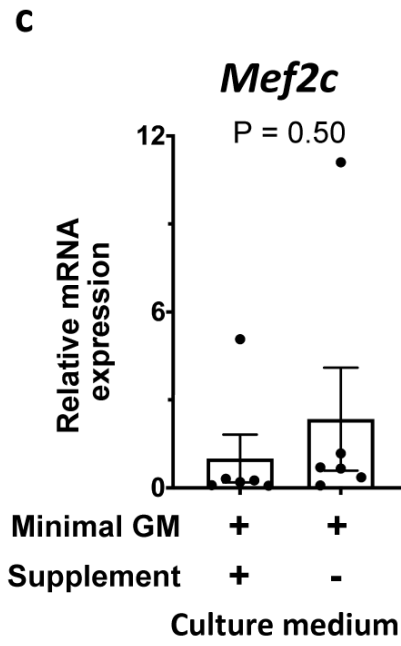
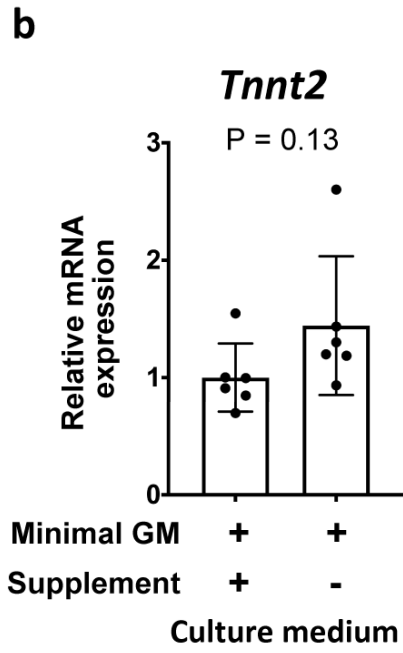
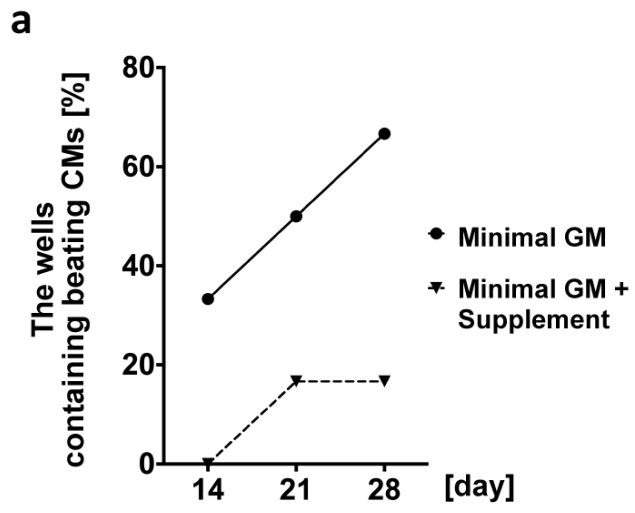
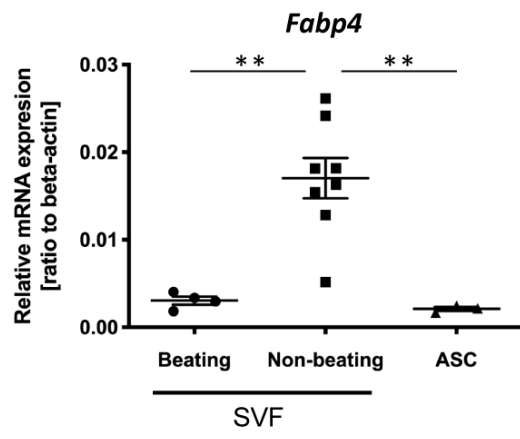
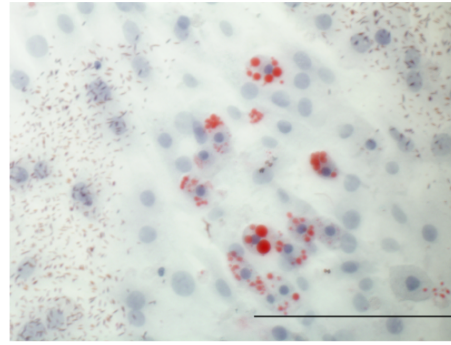
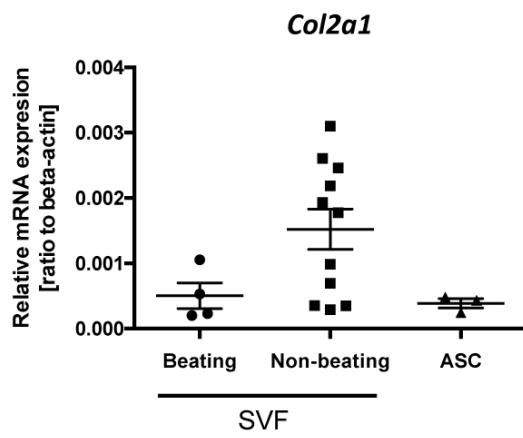


Fig.S1

**Figure S1. Transdifferentiation of SVF cells into spontaneously beating CMs in primary culture.** (a, b) The number of colonies and relative total RNA level of beating cells on day 28 after plating SVF cells (passage 0) or ASCs (passage 3). SVF, stromal vascular fraction (passage 0), ASCs, adipose tissue-derived stem cells (passage 3) obtained by subculturing SVF cells three times. (c) mRNA level of *Tnnt2* determined by qRT-PCR on day 28. (d) Flow cytometry of the stem cell surface markers CD34, CD90, and CD140a in SVF cells (passage 0) and ASCs (passage 3) on day 28, respectively. Gray peak, isotype control. (e–l) mRNA levels of key genes in SVF cells and ASCs on day 28 determined by qRT-PCR. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  by *t*-test.



**Figure S2. Efficacy of the generation of beating SVFs in different growth media.** (a) Frequency of wells with growth of beating SVF clusters in our minimal growth medium (GM) versus the complete GM (minimal GM plus supplements including growth factors and cytokines; see Methods) developed by Planat-Benard *et al.* (2004), on days 14, 21, and 28 of SVF cultivation. (b, c) The relative gene expression of Tnnt2 (b) and Mef2c (c) in all SVF cells on day 28 of cultivation using minimal versus complete GM. The *p*-values were determined by *t*-test.

**a****b****c**

**Figure S3. Characteristics of SVF and ASCs that transdifferentiate into adipocyte and cartilage lineages. (a)** mRNA level of Fabp4 in beating and non-beating cells in SVF and ASC on day 28. **(b)** Oil Red O staining of non-beating cells in SVF on day 28. **(c)** mRNA level of Col2a1 in beating and non-beating cells in SVF and ASC on day 28. \*\*  $p < 0.01$  by ANOVA. Scale bar, 200  $\mu\text{m}$ .

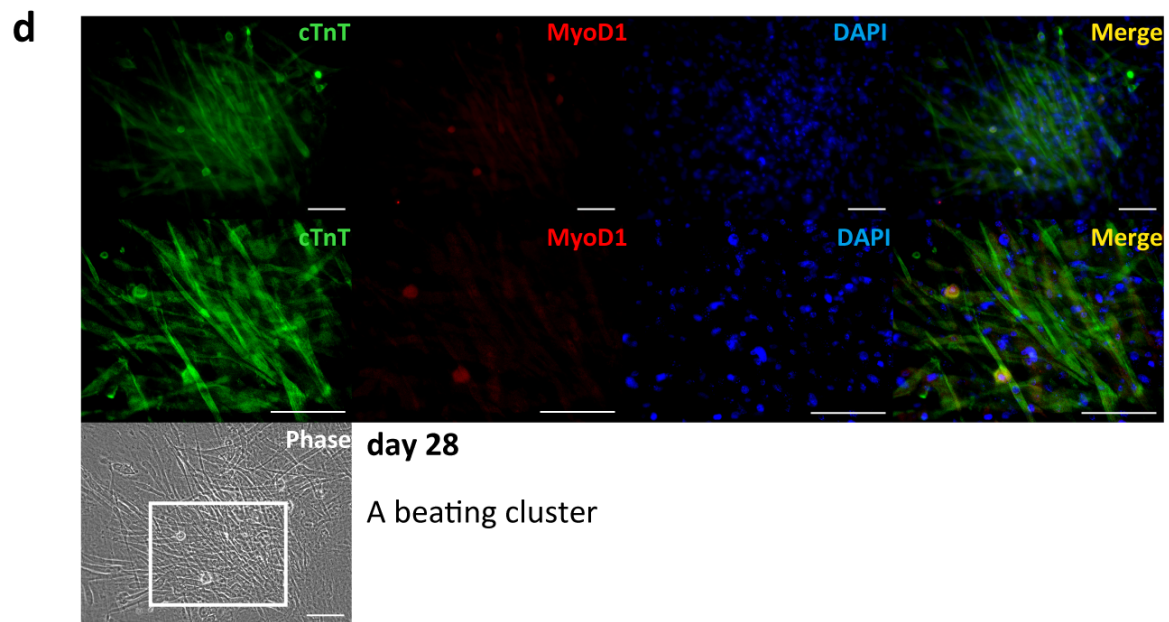
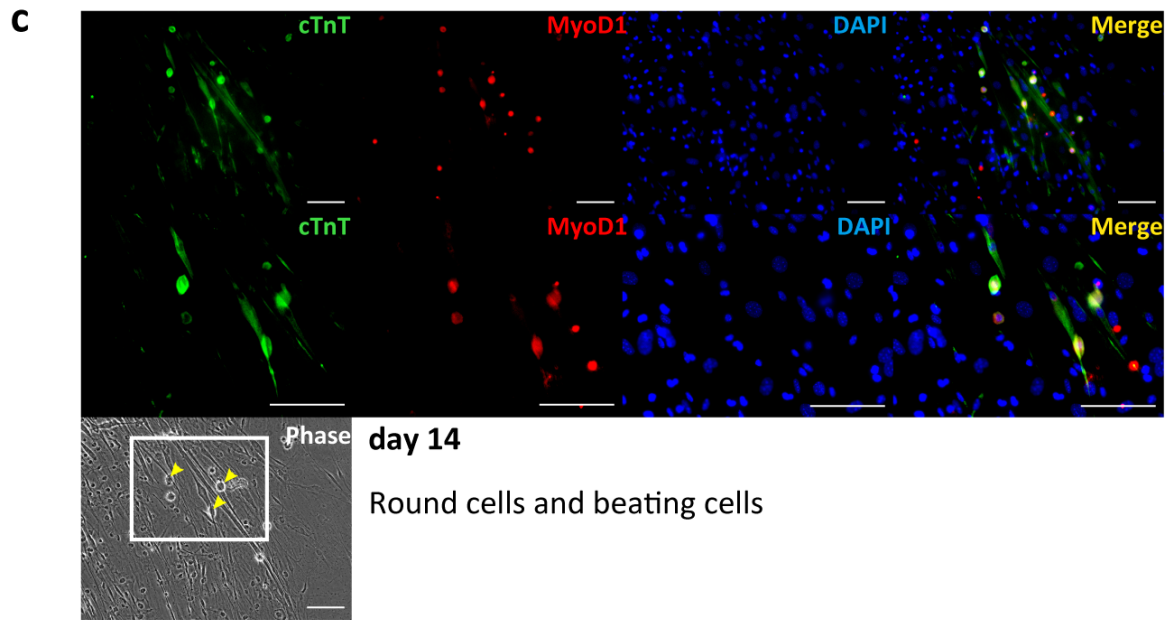
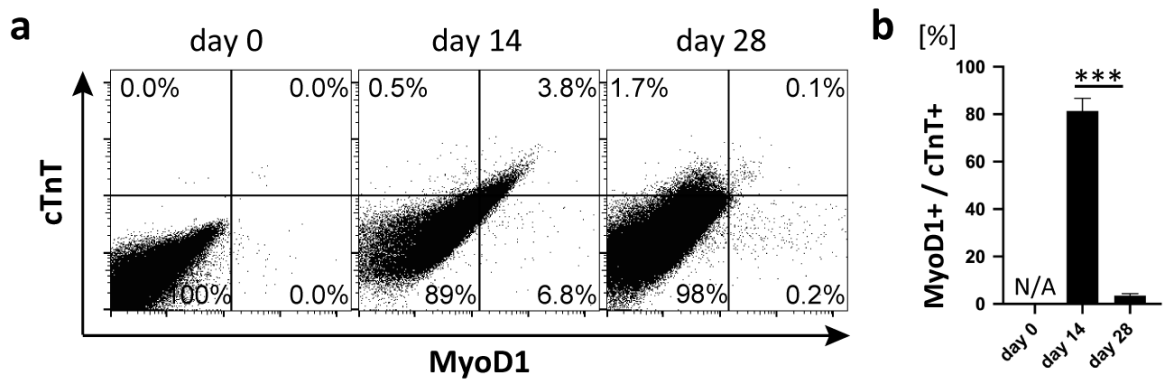


Fig.S4



**Figure S4. Gene and protein expression of MyoD1 and cardiac troponin T in SVF cells during primary culture.** (a) Flow cytometric analysis of SVF cells before (day 0) and after cultivating for 14 and 28 days. The cells were stained with APC-conjugated MyoD1 and FITC-conjugated cardiac troponin T (cTnT) antibodies. The number in each quadrant indicates the percentage of cells. (b) The ratios of the number of MyoD1-positive cells to the number of cTnT-positive cells on days 14 and 28. \*\*  $p < 0.001$  by *t*-test. (c) Representative immunofluorescence images of round cells and beating cells stained for cardiac troponin T (cTnT) and MyoD1 on day 14 after cultivating SVF cells. The arrowhead indicates a round cell. (d, e) Representative immunofluorescence images of beating (d) and non-beating (e) clusters stained for cTnT and MyoD1 on day 28 after cultivating SVF cells. White square, area of interest (shown above at a higher magnification). Scale bar, 100  $\mu\text{m}$ .

**Table S1. TaqMan assay ID of genes used in the TanMan qPCR**

Gene	TaqMan assay ID
Tnnt2	Mm01290256_m1
Myh6	Mm00440359_m1
Actc1	Mm01333821_m1
Myl2	Mm00440384_m1
Myl7	Mm00491655_m1
Hcn4	Mm01176086_m1
Hcn2	Mm00468538_m1
Gja1	Mm00439105_m1
Nppb	Mm01255770_g1
Nppa	Mm01255748_g1
Gata4	Mm00484689_m1
Mef2c	Mm01340842_m1
Tbx5	Mm00803518_m1
Nkx2-5	Mm01309813_s1
Fgf2	Mm00433287_m1
Kit	Mm00445212_m1
Cxcr4	Mm01996749_s1
Cxcl12	Mm00445553_m1
Fabp4	Mm00445878_m1
Col2a1	Mm01309565_m1

Genes	Catalog number
mACTB (VIC™)	4352341E

**Table S2. The accession IDs in NCBI's Gene Expression Omnibus of which data were used.**

<b>Platform</b>	GPL6246 [MoGene-1_0-st] Affymetrix Mouse Gene 1.0 ST Array [transcript (gene) version]
<b>Series</b>	<b>Sample</b>
GSE14414	GSM360098: heart_WT_rep1
GSE14414	GSM360099: heart_WT_rep2
GSE14414	GSM360100: heart_WT_rep3
GSE14414	GSM360108: embryo cardiomyocyte_rep1
GSE14414	GSM360109: embryo cardiomyocyte_rep2
GSE14414	GSM360110: embryo cardiomyocyte_rep3
GSE22292	GSM554758: neonatal cardiomyocyte_rep1
GSE22292	GSM554759: neonatal cardiomyocyte_rep2
GSE22292	GSM554760: neonatal cardiomyocyte_rep3
GSE24940	GSM613012: muscle1_rep1
GSE24940	GSM613013: muscle1_rep2
GSE24940	GSM613014: muscle1_rep3

## **Titles of the Supplementary videos**

**All of the videos were original and the speed was not altered.**

**Supplementary video S1.** Representative beating SVF on day 12 of a primary culture of Ad-SVF; related to Fig. 1a.

**Supplementary video S2.** Representative beating SVF on day 14 of a primary culture of Ad-SVF; related to Fig. 1b.

**Supplementary video S3.** Representative beating SVF on day 20 of a primary culture of Ad-SVF (low magnification); related to Fig. 1c.

**Supplementary video S4.** Representative beating SVF on day 20 of a primary culture of Ad-SVF (high magnification); related to Fig. 1c.

**Supplementary video S5.** Representative beating SVF on day 28 of a primary culture of Ad-SVF (low magnification); related to Fig. 1d.

**Supplementary video S6.** Representative beating SVF on day 28 of a primary culture of Ad-SVF (High magnification); related to Fig. 1d.

**Supplementary video S7.** Phase-contrast movie of a cluster of SVF beating cells on day 14; related to Fig. 1e.

**Supplementary video S8.** Intracellular calcium oscillation in round cells and elongated-tube-like cells on day 14 that corresponds to video 7; related to Fig. 1f.

**Supplementary video S9.** Intracellular calcium oscillation in the cluster of beating SVF cells on day 28; related to Fig. 1h and 1i.

**Supplementary video S10.** Phase-contrast movie of beating SVF cell co-expressing cardiac troponin T with Mef2c; related to Fig. 6c.